Paulo Célio Alves
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Editors

Lagomorph Biology

Evolution, Ecology, and Conservation

Springer

Paulo C. Alves   Nuno Ferrand   Klaus Hackländer (Eds.)

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With 78 Figures, 3 in Color, and 21 Tables

Springer

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Preface

The idea to publish this book was born at the 2nd World Lagomorph Conference in Vairão, Portugal, in July 2004. Twenty-five years after the 1st World Lagomorph Conference, a vast amount of literature on this mammalian taxon had been published and it was time to bring together researchers from all over the world to present and discuss the latest knowledge about hares, rabbits, and pikas. After the first announcement for the meeting we received several encouraging e-mails, among them a very special one:

"We want to offer you and your committee congratulations and best wishes on holding the 2nd World Lagomorph Conference in Portugal 25 years after the 1st World Lagomorph Conference in Canada in 1979. The world's lagomorph workers are a large, widely separated group with broadly different research programs, but, as Guelph showed, form a strongly united group with common interests when they get together like this. A statement on the present position of world lagomorphs, and its publication, are both timely and immensely valuable.

Good luck to you. Yours fraternally,

Ken Myers, Australia, and Charles McInnes, Canada,
Convenors, 1st World Lagomorph Conference, Guelph, Canada, 1979"

The approximately 150 participants from over 27 countries and all continents created a great atmosphere in Vairão, and the discussions promoted by the numerous talks, posters, and workshops certainly increased considerably the enthusiasm in working in such a fascinating mammal group. At the end of the conference, nearly all colleagues recognized that there is an urgent need to provide a comprehensive overview on the current fields in Lagomorph research. Therefore, we asked all participants in Vairão to submit manuscripts on their scientific work. As we did not intend to produce a classical conference proceedings, we also encouraged the participants to write reviews on some important issues, and we then made a peer-reviewed selection of the various topics. This book contains 26 contributions covering different topics in Lagomorph biology. All chapters are organized into sections. The first chapter, "Introduction to the Lagomorpha" by Joseph

Chapman and John Flux is an updated version of the introduction done in 1981 (Myers and McInnes 1981, Proceedings of the World Lagomorph Conference held in Guelph, Ontario, August 1979, University of Guelph). Here the reader will be introduced to the main aspects of taxonomy, ecology, behaviour, and conservation in Lagomorphs. In addition, the authors present a historical overview on the research activities in these fields.

Then, four chapters will highlight some reviews and aspects in *Palaeontology and Evolution* to understand how humans have contributed to the nearly world-wide distribution of rabbits and hares and to demonstrate the state-of-the-art in modern phylogenetics and phylogeography. In the section *Population Ecology and Dynamics* readers will gain insight into the general role of Lagomorphs in the ecosystems, how environmental conditions affect populations, and how scientists try to uncover the mechanisms of population dynamics. In the section that follows, *Physiology and Behaviour*, we will get deeper into Lagomorph biology and learn about reproduction and mother–young relationships as well as interspecific competition between Lagomorphs. Another major factor affecting population dynamics is covered in the section *Diseases*, in which the authors present all the current knowledge on important viral diseases and why these viruses are of interest for conservation and pest control. As some Lagomorph species are highly endangered while others are treated as pest, in the section *Conservation and Management* a number of chapters will contribute to a better understanding of this apparently antagonistic treatment of Lagomorph species. Here, readers will be updated on the current conservation status of each species and learn why some selected species should be managed and how this should be done.

We close the book by highlighting those aspects of Lagomorph biology that have been improved over the last 25 years, and then follow with some open aspects that should be, in our opinion, developed in the future.

This book evolved with the help of many colleagues who assisted in reviewing the submitted manuscripts. We want to thank Vilmos Altbäcker, David Hik, Karen Hodges, Robyn Hudson, Diana Pöttschacher, Toni Lavazza, John Litvaitis, Jerôme Letty, Pierre Mein, Dennis Murray, Greg Mutze, Benôist Schaal, Ferdy Rühe, Thomas Ruf, Andrew Smith, Becs Smith, Phil Stott, Jean-Denis Vigne, and Dan Williams. In addition, we appreciate the support of the team at Springer Heidelberg, especially Ursula Gramm and Dieter Czeschlik.

Paulo C. Alves, Nuno Ferrand, and Klaus Hackländer

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Introduction to the Lagomorpha

JOSEPH A. CHAPMAN[1] AND JOHN E.C. FLUX[2,*]

Introduction

Lagomorphs (pikas, rabbits and hares) are widespread, being native or introduced on all continents except Antarctica. They are all herbivores, occupying an unusual size range between the rodents, "small mammals", and ungulates, "large mammals". Pikas weigh 75–290 g, rabbits 1–4 kg, and hares 2–5 kg. Despite the small number of extant species relative to rodents, lagomorphs are very successful, occurring from sea level up to over 5,000 m, from the equator to 80 N, and in diverse habitats including tundra, steppe, swamp, and tropical forest. Some species have extremely narrow habitat tolerance, for example the pygmy rabbit (*Brachylagus idahoensis*) requires dense sagebrush, the riverine rabbit (*Bunolagus monticularis*) is restricted to Karoo floodplain vegetation, and the volcano rabbit (*Romerolagus diazi*) to zacaton grassland. On the other hand, the tapeti (*Sylvilagus brasiliensis*) occurs from alpine grassland at the snowline to dense equatorial forest on the Amazon, and some hares (*Lepus* spp.) occupy vast areas. According to the latest review by Hoffmann and Smith (in press), there are about 91 living species, including 30 pikas, 32 hares and 29 rabbits (see Fig. 1, families and genera of living lagomorphs).

Taxonomy

Lagomorphs were classified as rodents until the Order Lagomorpha was recognized in 1912. Anatomically, they can be separated from rodents by a second set of incisors (peg teeth) located directly behind the upper front incisors. The pikas (Ochotonidae) have 26 teeth (dental formula i. 2/1, c. 0/0, p. 3/2, m. 2/3) and rabbits and hares (Leporidae) have 28 teeth (i. 2/1, c. 0/0, p. 3/2, m. 3/3.). All lagomorphs have an elongated rostrum of the skull, fenestrated to a latticework of bone to reduce weight, a feature unique to the order. The soles of the feet are hairy, providing insulation against cold and extreme heat, and a good grip on most surfaces.

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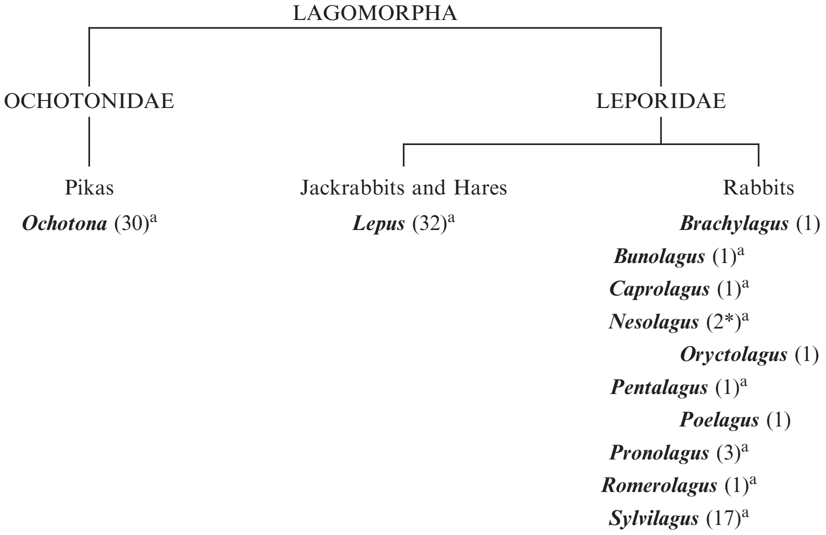

Fig. 1 Families and genera of living lagomorphs. After each genus is indicated the number of species
[a]Genera with at least one endangered species
*Includes the recently described species in Vietnam, *N. timminsi*

Pikas are in the monotypic family Ochotonidae, which includes a single genus, *Ochotona*. They have a long and distinct ancestry separate from the leporids since at least the Eocene (50 million years ago). All are highly adapted to their alpine or steppe environments, both morphologically and behaviorally. Taxonomically, they are a difficult group, partly because of speciation on separate mountain tops or other remote places where recognition is probably by sound rather than sight, and partly because the genus is geologically old, leading to a proliferation of relict species, but only seven recent species have a fossil record (Erbajeva 1997).

The earliest fossil ancestor of hares and rabbits (Leporidae) lived in Mongolia 55 million years ago (Asher et al. 2005). There are 11 genera (ten if the pygmy rabbit (*Brachylagus*) is included in *Sylvilagus*). Most are monotypic: *Brachylagus, Bunolagus* (the riverine rabbit), *Caprolagus* (the hispid hare), *Oryctolagus* (the European rabbit), *Pentalagus* (the Amami rabbit), *Poelagus* (the Bunyoro rabbit), and *Romerolagus* (the volcano rabbit); but *Nesolagus* has two species, *Pronolagus* three (the red rock rabbits), *Sylvilagus* 17 (the cottontail rabbits) and *Lepus* 32 (the true hares and jackrabbits). *Lepus* is another difficult group taxonomically, in this case because the group is recent and has spread on open land following forest clearance by ice ages and man; and specific recognition is probably chiefly by scent, which is not a normal taxonomic character. The world total reached 71 in 1900, but was reduced to 21 in 1960. Since then it has increased again to about 32; even in well-studied Europe, the number rose from two to the current six (Niethammer and Krapp 2003).

These changes in the number of lagomorph species are illustrated in the editions of "Mammal Species of the World": in 1982 there were 18 pikas, 24 rabbits, and 19 hares (Honacki et al. 1982); in 1993, 25 pikas, 24 rabbits, and 30 hares (Wilson and Reeder 1993); and currently, 30 pikas, 29 rabbits, and 32 hares (Wilson and Reeder 2005). Most of the increase is a result of splitting the existing taxa, but it is remarkable that a completely new species (Annamite rabbit, *Nesolagus timminsi*), which is as distinctive as the Sumatran striped rabbit (*Nesolagus netscheri*), could remain unknown almost into the 21st century (Surridge et al. 1999; Can et al. 2001).

General Adaptations

All the lagomorphs have adapted for quick movement in order to escape predators. Pikas and most of the rabbits sprint to thick cover or burrows, but hares are usually long-distance runners that can outlast their pursuers; some reach 80 km/h, and can maintain 50 km/h for hours. Unlike the pikas, which tend to be diurnal, hares have large eyes suited to their crepuscular or nocturnal habits. Young hares are born fully furred and with open eyes; in contrast, rabbits are naked and blind at birth, and have to be cared for by the mother for 2–3 weeks.

The lagomorphs with the greatest natural distributions (holarctic) are the pikas and hares, although more evolutionary diversity is found in rabbits. The New World cottontails, with 17 species, are the most diverse, and include even semi-aquatic forms like the swamp rabbit (*Sylvilagus aquaticus*). The European rabbit (*O. cuniculus*) is now the most widespread, but this is due to repeated liberations and domestication; historically it was restricted to the Iberian Peninsula, although fossils show a southern European distribution prior to the ice ages. Other genera such as *Pentalagus, Bunolagus, Caprolagus, Romerolagus* and *Nesolagus* appear to be "primitive" evolutionary branches now confined to islands or small areas of special habitat; many of these unusual and little-known forms are endangered (Smith 2008, this book).

Economically and scientifically, lagomorphs are one of the most important mammal groups. Domestic rabbits are a major food resource, and they are also key laboratory animals in medical research. Many lagomorphs are also valued game animals, while others are pests of national significance; both aspects have resulted in generous funding for ecological research programs globally.

Role in Ecosystems

Lagomorphs make up the base of many predator/prey systems. Their intermediate size and great abundance allows them to support a community of small to medium-sized predators such as weasels, foxes, cats, civets, and a large number of birds of prey. Thus the conservation status of the European lynx and Spanish imperial eagle depend on the number of rabbits present, and these have declined dramatically following outbreaks of myxomatosis and Rabbit Haemorrhagic Disease (RHD) (Ferrer and Negro 2004).

Most lagomorphs are selected for high rates of reproduction. Their numbers are regulated by extrinsic and intrinsic factors and dispersal of both adults and young. They have evolved survival mechanisms suited to a wide variety of environments and situations, but being herbivores low on the food chain, their habitat must provide forage and adequate escape cover. Even so, annual mortality rates may reach 90% in many species. Such patterns are common to many game species and rabbits and hares are among the top species favored by hunters in Europe and America.

Reproductive patterns vary with latitude. In New World rabbits (*Sylvilagus* and *Romerolagus*), there is a direct correlation between latitude and litter size, with those in the north producing the largest litters in shorter breeding seasons (Conaway et al. 1974). In hares (*Lepus*), the same direct correlation between litter size and latitude and an inverse correlation with breeding season, results in an almost constant annual production of young per female for all species (Flux 1981), a phenomenon which remains to be explained.

Rabbits in northern latitudes also have shorter gestation periods. This allows northern rabbits to produce more litters in the most favorable weather. Conversely, it is an advantage for tropical rabbits to have longer gestations because more fully developed young are better able to avoid predators and fend for themselves (Chapman 1984). Old World hares do not seem to follow this pattern; *Lepus timidus* has a longer gestation than *Lepus europaeus*, but young hares (*L. capensis* and *Lepus crawshayi*) on the equator in Kenya are almost as heavy at birth as young European hares, despite the mothers weighing half as much (Flux 1981). In compensation, the litter size is smaller and the breeding season longer.

Lagomorph Behavior

The behavior of the European rabbit has been studied more than any other lagomorph, but it should be remembered that much of this work has been done in Australia and the United Kingdom, where rabbit control is of great importance. These rabbits are now thought to be feral populations derived from escaped domestic stock, which may be atypical in lacking intrinsic population control behavior (Flux 2001). This may explain the tendency for *O. cuniculus* to reach a biomass ten times higher than any other lagomorph and overeat its food supply, causing major agricultural problems. There has been considerable behavioral work on pikas in Japan, the United States, and the Soviet countries, mostly purely scientific with little emphasis on applied problems, except in China (see Smith et al. 1990). By comparison, there has been little work on cottontails, jackrabbits, and most other genera.

Cottontail males form breeding groups around several dominant females, choosing areas within the habitat that have better food and cover (Marsden and Holler 1964). Linear dominance hierarchies for females are not well defined, but dominance of unreceptive females over males during nest building, parturition or nursing is important in avoiding detrimental male harassment. The closest thing to courtship behavior in cottontails is a dash and jump sequence and related displays. Swamp rabbits (*S. aquaticus*) are territorial, but Eastern cottontails (*Sylvilagus floridanus*) appear to defend only the area around a breeding female. Vocalizations are reported for swamp rabbits, brush rabbits, pygmy rabbits, and mating volcano rabbits (Tefft and Chapman 1987).

Pikas have behavior patterns that are remarkably similar throughout their range and are aligned to habitat: pika species that live in rock screes at low, steady densities, are highly territorial and pugnacious, long-lived, with low fecundity; pikas that live in the steppe make burrows in which they live at high density, often in family groups. They are social, short-lived, and have high fecundity (Smith 2008, this book). They are the only lagomorphs with highly developed vocalization and they store food for winter in "hay piles", which are vigorously defended.

North American jackrabbits and other hares have very similar behavior while mating, which involves fighting between males to establish dominance (boxing) and between an unreceptive female and a male or males (rebuff). Both boxing and rebuff may be followed by vigorous chasing, but copulation follows only if the female, which is almost invariably dominant over the male, is receptive (Lechleitner 1958; Schneider 1978). "Ownership" of a food resource, or of a female, does not confer any advantage in dominance in European hares (Holley 1986). Vocalizations seem restricted to a grunting call by females to their young and loud screams when caught by a predator.

All of the lagomorphs have a digestive system involving coprophagy (the re-ingestion of soft feces to increase the nitrogen content of the diet) and some even eat hard feces if food is scarce (Hirakawa 2001). They also groom extensively; licking the fur, cleaning the feet, and dusting. In heavy rain, water is shaken away and the feet are kicked to dry them.

Lagomorph Conservation and Management

On a world scale, conservation and management of lagomorphs has included: (1) habitat management, (2) stocking programs, (3) harvest, (4) control and (5) protection. Habitat management has been used chiefly to increase stocks of animals for game shooting. Cottontail habitat in North America entails the creation of disturbed, successional, and transitional vegetation for food and cover by planting, clearing or burning; old pasture is one of the best habitats.

In Europe, there is a long tradition of managing estates for game, hares and deer being the most important mammals, but limited shooting seasons and provision of food in winter were the main management tools. Rabbits were highly valued and kept in elaborate warrens from their introduction about 1200 A.D. until the 19th century when increasing numbers and damage to agriculture changed their status to pests, at least in northern Europe.

Stocking programs have virtually been worldwide, beginning over 2,000 years ago, and often with undesirable results. The most notorious was probably the introduction of European rabbits to Australia, New Zealand, and South America, where many farms were ruined; others include the establishment of European hares in Canada, North and South America, Australia, New Zealand, and Russia. Hunters liberated cottontails in Europe to take the place of European rabbits when numbers were reduced by myxomatosis and RHD. Since cottontails can carry myxomatosis but are not adversely affected by it, they would be expected to replace the European rabbit rapidly, but this does not seem to have happened (Spagnesi and De Marinis 2002), perhaps because the cottontails have not adapted to new habitats (Rosin et al. 2008, this book). In North America, stocking cottontails for hunting has a long history with little regard for disease transmission or impact on native species (Chapman and Morgan 1973); the decline of *Sylvilagus transitionalis* in the 1920s led to massive introductions of *S. floridanus* for 30 years (in the 1930s Pennsylvania alone was importing 50,000 a year) and the impact on *S. transitionalis* populations was severe. The mechanism involved seems to be the better ability of *S. floridanus* to exploit small patches of habitat rather than interference competition (Litvaitis et al. 2008, this book). The introduction of *L. europaeus* into Italy now threatens the endemic hare, *Lepus corsicanus* (Trocchi and Riga 2001). The decline of European rabbit populations in the Iberian Peninsula is being tackled with restocking programs by hunters and conservationists (Moreno et al. 2004).

The harvest of lagomorphs has a history dating back 120,000 years associated with their value for food and fur. Pikas are generally too small to be a significant food source, but in Russia, as many as 14,000 skins a year were used for high-quality felt until the 1950s (Smith et al. 1990). The market in domestic rabbits (*O. cuniculus*) is enormous and too well known to require comment; wild populations in Europe, South America, Australia, and New Zealand have also been exploited. For example, New Zealand exported an average of 12 million a year to Britain between 1900 and 1945 (Flux 1997). Among hares, the largest harvests are probably of *Lepus americanus, Lepus californicus* and *L. europaeus*; 70 million European hares a year were exported from South America to Europe in the 1980s (Tume 2000).

The control of lagomorphs has usually been a result of conflict with agriculture, and dates back to at least 63 B.C. (Barrett-Hamilton 1912). In North America, problems include snipping or girdling alpine trees by pikas and snowshoe hares, and farm damage by cottontails or, more often, jackrabbits; in some western states, jackrabbits have occasionally become rampant pests requiring widespread control. Four species of pika have been designated as pests in Asia because they damaged crops and valuable trees, and competed with domestic stock on rangeland (Smith et al. 1990). However, the most serious problems have undoubtedly followed the introduction of European rabbits (Thompson and King 1994).

Protection of lagomorphs also dates back over 2,500 years because of their value as game, and continues in North America and Europe today. Since 1978, when the Lagomorph Group of IUCN was established, more systematic attention has been paid to threatened and endangered species (usually those with very restricted ranges – See Chapman and Flux 1990) but the continuing decline shown in game bags of even widespread species like the European hare in Europe is of increasing concern (review in Smith et al. 2005).

Trends in Lagomorph Research

In the 25 years since the first World Lagomorph Conference in 1979, there has been much change in the direction of research. Some of this results from new tools, like DNA analysis, satellite imagery, GPS tracking, night-vision equipment and video filming, and the enormous increase in the computing power available. Some is a result of politics; most countries have adopted research management along business lines, with a whole new jargon of tightly focused outputs, promoting applied research at the expense of basic science. Some problems have risen to prominence such as climate change, loss of biodiversity, and novel diseases like RHD and European Hare Syndrome (EBHS).

In an attempt to measure these changes, I compared the topics covered at the 1979 meeting (Myers and MacInnes 1981) with the present one. There were 93 papers in 1979 and 170 in 2004, but the latter were more specialized, so the number of topics covered increased only from 198 to 272. The biggest decreases since 1979 were in population dynamics (15 to 8%) and general life history (8 to 1%). There were also declines in papers dealing with behavior (13 to 10%), reproduction (9 to 6%), control (8 to 5%) and parasites and predators (8 to 6%). Papers on food habits remained constant at 14%, and those on conservation increased very slightly (from 9 to 10%). However, there was a noticeable increase of interest in diseases (5 to 8%) reflecting work on calicivirus, and in management (2 to 10%), probably a local response to the importance of rabbits in the Mediterranean ecosystem. Finally, there was the obvious expansion of taxonomy (6 to 13%) and population genetics (4 to 10%) associated with modern DNA techniques, giving exciting new results.

On the other hand, many problems remain unsolved. We still have no consensus on what regulates populations; what causes the 10-year cycle in snowshoe hares; how best to control pest lagomorphs, or how to increase the number of desirable ones. In taxonomy, we seem as far from a definitive list of species as ever before, despite the increasingly sophisticated techniques available: even the concept of what constitutes a species remains unclear. Yet this is normal in science, where every question, like a tired hare, raises another to take its place.

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Palaeontology and Evolution

Prehistoric and Historic Artificial Dispersal of Lagomorphs on the Mediterranean Islands

Marco Masseti[1] and Anna M. De Marinis[2,*]

Introduction

The current Mediterranean fauna is a result of the interactions of diverse factors, primarily the multiple biogeographical origin of the species, Quaternary climatic changes (which produced a repeated turnover of biota) and Late Pleistocene-Holocene human-induced habitat modifications, including hunting and Holocene introductions of a variety of allochthonous continental taxa (Masseti 1998, 2002). Apart from sporadic cases, the complete absence of endemic species from the extant mammalian fauna of the Mediterranean islands is quite surprising. In the majority of the cases, in fact, the existing populations of non-flying terrestrial mammals display undoubtedly a homogeneous composition of elements, predominantly revealing a continental origin (Alcover 1980; Sanders and Reumer 1984; Vigne 1992, 1993; Blondel and Vigne 1993; Masseti 1993, 1998). To assess the range of the original insular distribution of the different species in the Mediterranean region, earlier chronologies prior to the Neolithisation should be considered, after which improved human seafaring skills and the established commercial networks between countries enabled the artificial exportation even of wild animals, together with those already involved in the process of domestication (Masseti 1998; Lorenzini et al. 2002). Recent archaeological investigation indicates that the first transfers of allochthonous faunal elements were carried out subsequent to early Neolithic times, as documented by the discovery of the Pre-Pottery Neolithic site of Shillourokambos on the island of Cyprus (end of the 9th–8th millennium B.C.) (Guilaine et al. 1996, 2000; Cucchi et al. 2002; Vigne et al. 2003). It seems that from these times on, man began to bring with him the animals he needed as economic supplies for the colonisation of new geographical areas. One of the faunal categories much exploited in this sense is represented by the Lagomorph family, originally represented among the Late Quaternary endemic faunal horizons of the Mediterranean islands only by one species, the ochotonid *Prolagus sardus* (Sardinian pika) (Wagner 1829), native and exclusive of the Upper Pleistocene-Holocene of Corsica and Sardinia. In the light of

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archaeozoological evidence, this endemic lagomorph survived on the two islands up to very recent times, probably coinciding with the Iron Age (Delussu 2000; Wilkens and Delussu 2003), or even up to Roman times (cf. Vigne and Valladas 1996; Vigne 1997). According to the Greek historian Polybius (3rd–2nd century B.C.), the island of Corsica of his time was not characterized by the occurrence of any species of hare, but was instead inhabited by this Sardinian pika, locally called the *kyniklos*. Polybius observed that "*... when seen from a distance* [it] *looks like a small hare, but when captured it differs much from a hare both in appearance and taste. It lives for the most part under the ground*" (*The Histories*, XII: 3.8–4.6 in Paton 1925). However, evidence shows that the extant lagomorphs of the Mediterranean islands exhibit an apparently undifferentiated continental origin. The occurrence of these continental forms on all the islands seems to be linked essentially to the introduction by man during the Holocene.

In the present work, we reviewed the earliest recorded introductions of the Apennine hare, *Lepus corsicanus* (De Winton, 1898), the European hare, *Lepus europaeus* (Pallas, 1778), the Iberian hare, *Lepus granatensis* (Rosenhauer, 1856), the Sardinian hare, *Lepus ("capensis") mediterraneus* (Wagner, 1841), the Mountain hare *Lepus timidus* (Linnaeus, 1758), the European rabbit, *Oryctolagus cuniculus* (Linnaeus 1758), and the Nearctic Eastern cottontail, *Sylvilagus floridanus* (Allen 1890) onto the Mediterranean islands in the light of recent archaeological investigations. Moreover, we updated their extant insular distribution considering the fact that they have been the object of artificial translocations also carried out in very recent times.

Materials and Methods

Data on lagomorph distribution were gathered from: i) literature; ii) field observations (sightings and dead specimens) carried out on the Tuscan Archipelago (Italy), Sardinia, circum-Sicilian and Maltese Archipelagos, Ionian and Aegean Islands, Crete and Cyprus within the last 10 years; iii) museum collections (Natural History Museum, London; Muséum National d'Histoire Naturelle, Paris; Zoological Museum, University of Florence; Natural History Museum, Crete; Natural History Museum, Vienna; Research Institute and Natural History Museum Senckenberg, Frankfurt am Main; Alexander König Zoological Museum, Bonn; Zoological Museum, University of Patras; Zoological Museum, University of Athens and The Bavarian State Collection of Zoology, Munich). The area of each island where lagomorphs are present today has been recorded.

Islands for Hares

Apennine hare, *L. corsicanus* De Winton, 1898. Endemic to the south-central Italian Peninsula and Sicily (Palacios 1996; Riga et al. 2003), this taxon was described by De Winton in 1898 on the basis of the examination of some specimens obtained from Corsica. This fact has given rise to the unproven assumption that this hare was native to Corsica. Hares, however, never figured among the fossil horizons of the large Mediterranean island, where *L. corsicanus* has been possibly introduced shortly before the 16th century A.D. as documented by historical evidence (Vigne 1988a, 1990, 1992, 1999; Fig. 1). The Greek historian Polybius, for example, did not mention the occurrence of any type of hares among the Corsican mammalian fauna of his time (*The Histories,* XII: 3.8–4.6 in Paton 1925). The Apennine hare could have been originally present in the south-central Italian Peninsula and Sicily as an endemic relict population divergent from the hares dispersed in northern Italy, and possibly of a more ancient origin. In fact, these territories harbour several taxa of mammals that differ substantially from their northern counterparts, i.e., the Roman mole, the Apennine chamois, the red fox along with the snow vole, and the Italian roe deer (Frati et al. 1998; Lorenzini et al. 2002). Recent morphometric (Palacios 1996; Lo Valvo et al. 1997; Riga et al. 2001, 2003) and genetic (Pierpaoli et al. 1999, 2003) analyses have confirmed the taxonomic validity of *L. corsicanus*, attesting at the same time its present occurrence in south-central Italy, on Corsica and Sicily (Angelici and Luiselli 2001).

The European hare, *L. europaeus* Pallas, 1778. The European hare is a continental faunistic element characteristic of the Palaearctic Biogeographical Region (cf. Corbet 1978). There is no fully convincing evidence for the importation of this hare on any islands before the Early Bronze Age (Masseti 2003a; Fig. 1). In the light of recent archaeozoological evidence, in fact, the earliest relevant documentation appears to come from the eastern Mediterranean basin from the islands of Crete (Reese 1995; Jarman 1996) and Amorgos (Trantalidou 2000). The existence of the lagomorph in insular environments has been documented by classical authors, such as Homer (*Odyssey*, IX, 116–124) and others. Xenophon (5th century B.C.), in his *Kinegeticon*

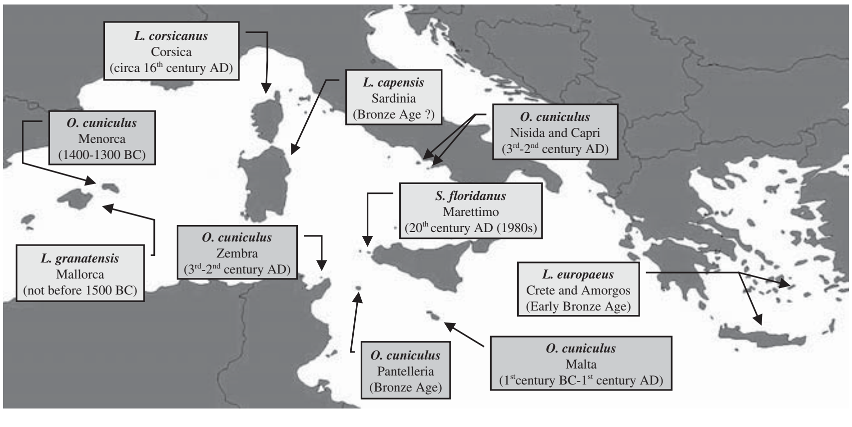

Fig. 1 Earliest recorded introductions of hares, European rabbit, and Eastern cottontail onto the Mediterranean islands

(24–26), observed that hares were particularly abundant on islands because predators such as foxes and eagles occur less frequently than on the mainland. The ancient practice of releasing hares on islands, considering the latter as natural enclosures, was maintained up to historical times in the *leporaria* of the Roman period (Varro, *De re rustica*, III, 12, 1; Bodson 1978*)* and the Middle Ages (Masseti 2003a). The European hare presently occurs on about 50 islands from the Central Mediterranean basin to Cyprus (cf. Averianov et al. 2003). Most of these islands (93%) are localized in the eastern basin and 71% are larger than 50 km^2. In the second half of the 20th century, the introduction of the species on Sardinia and Sicily has failed (Lo Valvo et al. 1997). The European hare has become extinct recently on at least five Greek islands: Sifnos (western Aegean Islands), Delos (Cycladic Archipelago), Paximada (Dionysades Islands), Astypalaia (Dodecanese), and Lemnos (north-eastern Aegean Islands). On the other Aegean islands, populations of European hare of ancient and/or modern introductions are today reported from several islands, such as Milos, Naxos, Amorgos, Crete, Karpathos, Rhodes, Ikaria, Samos, and others (Masseti 2003a).

Throughout most of the 19th and 20th centuries, however, there was a widespread practice among scientific explorers to bring home an excessive number of subspecies from their explorations of the Mediterranean islands. These authors often classified many of the insular populations as geographic forms, almost entirely on the basis of arbitrary criteria and the examination of scattered materials. Based on the data given in literature, the various subspecies of the insular hares are distinguished by the coat patterns and by the size of the body and skull. Therefore it is logical that this led to a multiplication of forms which now, however, demand better taxonomic and genetic definition. In this respect, we should therefore consider the extant taxonomic treatment on the part of the international scientific community of the lagomorphs of the Eastern Mediterranean islands, such as *L. e. creticus* Barrett-Hamilton, 1903, from Cephalonia (Miller 1912), Crete (Bate 1906 and 1913), and several small islets off Crete, including Gavdos and the Dionysades Islands (Niethammer 1992), *L. e. ghigii* (De Beaux, 1927), from Astypalaia (De Beaux 1927 and 1929), *L. e. carpathous* (De Beaux, 1929), from Karpathos (De Beaux 1929), *L. e. rhodius* (Festa, 1914), from Rhodes (De Beaux 1929; von Wettstein 1942), and *L. e. cyprius* (Barrett-Hamilton, 1903), from Cyprus (Ellerman and Morrison-Scott 1952).

Iberian hare, *L. granatensis* Rosenhauer, 1856. Endemic to the Iberian Peninsula (Palacios 1983), the Iberian hare is distributed throughout most of the territories of Portugal and Spain and also occurring at present on the Balearic island of Mallorca (Alcover 1988; Garcia-Perea and Gisbert 1999; Alves and Niethammer 2003). It was also formerly dispersed on Ibiza, where it is now extinct (Garcia-Perea and Gisbert 1999). Archaeological evidence documents the appearance of hares on the Balearics not before the Talayot culture (Alcover 1988), which flourished on these islands from 1500 B.C. to 500 B.C. (cf. Pericot Garcia 1972; Fig. 1). The earliest remains of the hare have been provided by the excavation of the site of S'Illot, on Mallorca (Uerpmann 1971). In very recent times, the Iberian hare was also introduced onto Corsica, where there is however no evidence that the species established a population (Garcia-Perea and Gisbert 1999).

Sardinian hare, *L. ("capensis") mediterraneus* Wagner, 1841. In the light of recent genetic and morphometric research, the hare dispersed presently on Sardinia belongs to the subspecies *L. ("capensis") mediterraneus* (Trocchi et al. 2003). Thus, the geographical origin of this insular population might be to refer to the southern continental shores of the Mediterranean basin, from where hares might have been introduced onto Sardinia in ancient times. In fact, there is data that show that Sardinian hares are closely related to hares in northern Africa (Pierpaoli et al. 1999; Alves et al. 2003). There is, however, no archaeozoological evidence of the importation of hares on the large Mediterranean island prior to the Bronze Age (Fig. 1). In fact, the discovery of a tibia of a hare at the site of Sierra Nedda (Sorso, Sassari) attests the occurrence of the lagomorph on the island for the first time (Wilkens 2000). Unfortunately, this piece comes from a level which is disturbed by the presence of black-figure pottery and hence its dating to the Bronze Age is not certain (cf. Rovina 1986), even though the appearance of the hare on Sardinia is of a period that is quite early (Wilkens and Delussu 2003).

Mountain hare, *L. timidus* L., 1758. This taxon is characteristic of the northern Palaearctic Region (Sulkava 1999). At lower European latitudes, the natural distribution of this hare comprises only the Alpine mountain arch. The species seems to have been also reported from Pleistocene insular fossil deposits, such as Grotta dei Colombi, on the island of Palmaria (La Spezia, north-western Italy) (Regalia 1893; Cigna 1967). The appearance of the same taxon among the fossil fauna provided by the excavation of the Grotta di Reale (Porto Azzurro) on the island of Elba (Del Campana 1910; Malatesta 1950–1951) is to be referred to a Late Middle or Late Pleistocene chronology (cf. Rustioni and Mazza 1993). No evidence is, however, available for the occurrence of mountain hares on Mediterranean islands in historical times, although during the 19th and the 20th centuries the taxon *L. timidus* was often erroneously recorded from several islands of the Tuscan Archipelago (Lanza 1970), such as Pianosa (Zuccagni-Orlandini 1842), and Elba (Branchi 1839; Damiani 1923). These two latter islands are today characterised only by the occurrence of *L. europaeus*, introduced in the 20th century (De Marinis et al. 1996; Masseti 2003b).

Islands for Rabbits and Cottontails

European rabbit, *O. cuniculus* (Linnaeus, 1758). This species is regarded as a post-glacial endemism of the Iberian Peninsula (Rogers et al. 1994; Callou 2003; Kaetzke et al. 2003). Thus, the spread of this taxon is the most important migration in Western Europe from an ancestral area restricted to these territories. This spread resulted from exchanges between human societies from the prehistory until the early Middle Ages (Callou 2004; Fig. 1). The earliest recorded introductions of rabbits onto the Mediterranean islands date back to the second half of the 2nd millennium B.C. (1400–1300 B.C.) when this lagomorph was apparently imported to Menorca, in the Balearic Archipelago, by ancient settlers from the Iberian Peninsula (Sanders and Reumer 1984). In the Central Mediterranean basin the subfossil evidence for the introduction of the species dates to no earlier than the 3rd-2nd century A.D., since remains of rabbits have been provided by the archaeological exploration of the islands of Nisida and Capri, in the Gulf of Naples (Italy), and Zembra in Tunisia (Barrett-Hamilton 1912; Vigne 1988b; Albarella 1992; Flux and Fullagar 1992; Flux 1994; Callou 2003; Kaetzke et al. 2003). Recent archaeozoological evidence, however, suggests an earlier importation of the lagomorph, comprised between the 1st century B.C. and the 1st century A.D., in the case, for example, of the sanctuary of Juno at Tas Silg on Malta (J. De Grossi Mazzorin, personal communication) or, at least, to the Bronze Age for the site of Mursia on the island of Pantelleria, in the Sicilian Channel (Italy) (Wilkens 1987). Rabbits were probably not imported onto the Eastern Mediterranean islands before the end of the Classical period and/or the beginning of the Middle Ages. This can be also observed in the lack of toponyms in the Eastern Mediterranean basin. Among the other elements supporting the theory of a recent historical introduction of the rabbit onto the latter territories, we can consider the fact that Turkish people still refer to the species as the *ada tavscan* ("hare of the islands"). In the course of the present study it was possible to report the occurrence of the European rabbit from at least 150 islands, localized in the whole Mediterranean basin. Almost half of the islands (53%) are smaller than 5 km^2. The species is also present on islets as small as 0.02 km^2 (i.e., Isola delle Femmine, northern Sicily), therefore it is really a successful colonizer of the Mediterranean islands. In very recent times, the rabbit became extinct on a few islands of the western Mediterranean: i.e., Frioul, France; Mezzomare and Lavezzi, Corsica. On the two latter islands, it disappeared in 1977 due to the spread of myxomatosis (Flux and Fullagar 1992; Vigne et al. 1994). To preserve the insular biological diversity, the species has been subject of several attempts of eradication in the course of the last decade. The only case of eradication carried out in the Mediterranean basin concerned the archipelago of Columbretes, Spain (Jimenez 1994). A total of 175 individuals was eradicated from the island of Columbrete Grande (14 ha), where their former occurrence drastically changed the climax community *Chamaeropo humilis-Rhamnetum lycioidis*. It was possible to report the coexistence of the European hare and the European rabbit from several Greek islands, such as Aegina (Saronic Gulf), Gadaironisi, Theodoru, and Koufonisi (circum-Cretan islands), Paros, Andros, Milos, Leros and Tinos (Cyclades), Kos (Dodecanese), Lesbos and

Fourni (north-eastern Aegean Islands). Among these islands, only Theodorou and Gadaironisi, off the northern and the southern shores of Crete, respectively, are smaller than 5 km^2.

Eastern cottontail, *S. floridanus* (J.A. Allen, 1890). This is a native species to the Nearctic Region, where its natural distribution occurs over broad geographic provinces from Canada to north-western South America (Chapman et al. 1980; Chapman and Ceballos 1990). Eastern cottontails have been widely transplanted beyond the territories of their natural distribution in North America and Europe. In the Old World, the species has been introduced to France, where it is now extinct, and as well as to Italy (Lapini 1999). In Italy, the earliest evidence of the occurrence of the lagomorph dates back to 1966 (Doria 1991; Spagnesi 2002; Andreotti et al. 2001), and populations of cottontail are today dispersed in Piedmont, Friuli-Venezia Giulia and Tuscany (Lever 1985; Lapini 1999; Spagnesi 2002; Masseti 2003b). The only known attempt for the introduction of the species onto Mediterranean islands seems to have been performed on Marettimo, in the Egadi Archipelago (north-western Sicily) during the 1980s, but there is no evidence that these animals originated any local population (Zava 1992; Fig. 1).

Concluding Remarks

Together with the species already involved in the process of domestication, a variety of wild species were also brought onto the Mediterranean islands since Neolithic times, including shrews, hedgehogs, mice, spiny mice, dormice, foxes, weasels, martens, badgers, cats, deer and, last but not least, hares and rabbits. It is not immediately apparent why man should have wanted to introduce all these animals. This phenomenon can only be explained by considering each case individually (Masseti 1998). The evidence suggests, for example, that not all of the supposedly tamed and/or semi-domestic herbivores that were imported onto the islands may have been exploited in the same way, some of them being destined to breed in conditions of captivity and others in a free-ranging state while others still could have returned to the wild after their introduction, being subsequently hunted by man as fully established wild game (Masseti 1998). In fact, the appropriation to hunting ("*kynegetisation*") might be a component of Neolithisation, just as is animal domestication (Vigne 1993). While some species were suitable for breeding, others were suitable for hunting. This is one way of simplifying management problems, considering the islands as natural enclosures and allowing the wild animal populations to derive their food supply directly from the carrying capacity of the natural environment (Masseti 1998). This was apparently the only way to exploit the natural resources of territories that could not be cultivated and were generally unattractive economically (Masseti and Zava 2002a, 2002b). Some of the species more adaptable to the specific environmental conditions of even small and barren islands, such as hares, and later rabbits, were brought by sailors and released on islands so that they could breed and provide a source of fresh meat that would be readily available for the passengers of ships (Masseti 2003a). In fact, hares can survive on islands that do not exceed a surface area of 3–3.5 km^2. Rabbits can survive on even smaller islands than hares, as small as 0.02 km^2. For centuries, if not for millennia, several of the Mediterranean islands were better known for their richness in certain zoological species, most useful as a source for meat than for their faunal repertoire in general (Masseti 2002), and this is still reflected even now in the names of many of them, especially some of the smaller ones, such as Conejera (Spain), Isola dei Conigli (Italy), Conigliera (Tunisia), Levrera (Croatia), and others. Apart from the former introductions of hares and rabbits documented by the modern scientific research, during the last 4,000–3,500 years, this phenomenon could have taken place on a large number of other islands in the whole Mediterranean basin. Thus, the data reported in this work on the extant insular distribution of hares and rabbits may be still far from conclusive, especially in regard to their present occurrence on the islands of the Adriatic gulf, Ionian Sea, and the Aegean Sea. The invasion of Mediterranean islands by exotic taxa is currently viewed as one of the most important causes of the loss of biodiversity (Gippoliti and Amori 2002; Courchamp et al. 2003). Rabbits, for example, can damage vegetation, accelerate soil denudation and erosion, and can be ultimately responsible for the decline or extinction of several reptile and bird species (e.g., Courchamp et al. 2003; Chapuis et al. 2004; Genovesi 2005). Therefore, in regard to the vulnerability of the ecosystems of the Mediterranean islands, it would be advisable, from both a scientific and a cultural point of view, to seriously consider the eradication of the alien species of very recent invasion, as it was in the case of the rabbits of Columbrete Grande. But this leaves the question of how to treat the allochthonous populations of certified ancient anthropochorous origin that represent invaluable historic documents. The study and observation of these populations enable us to acquire fundamental information about the progress of man through the millennia of his process of civilisation; they therefore deserve to be protected, considering them in terms of a veritable "cultural heritage" (Masseti 2002).

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The Lagomorph Fossil Record and the Origin of the European Rabbit

Nieves Lopez-Martinez

Introduction

Lagomorphs are very prolific mammals with a rich fossil record, which particularly increased over the last years by washing and screening techniques for microfossil recovery. Fossil remains of lagomorphs have been extensively documented in the Old World and North America from Early Paleogene onwards (around 45 Ma). Lagomorph diversity is much larger in the fossil record than in the biosphere. Only 12 genera and about 75 lagomorph species are still living in recent times, most of them almost devoid of paleontological record. In contrast, around 75 genera and more than 230 species, most of them already extinct, are represented in the fossil record of Lagomorpha. The local faunas today rarely contain more than three sympatric lagomorphs, frequently just one or two taxa. Instead, up to eight lagomorph species coexisted in local paleofaunas. This pattern constitutes a rare case in the recorded history of organisms, since the fossil record even in well-represented groups contains a lower number of species than the biosphere. Only declining groups, such as brachiopods or perissodactyl mammals, show higher diversity in the past than in the present, which denotes that lagomorph lineages are also declining in recent times.

One of the most widespread living lagomorph species is the European rabbit *Oryctolagus cuniculus*, whose areal distribution has been greatly expanded and modified by anthropic dispersal. All living European rabbit's subspecies *O. c. cuniculus* (Germany), *O. c. algirus* (Algeria) and *O. c. huxleyii* (Madeira), have been described from apocoric localities (i.e., lying outside the primitive range of the species; Gibb 1990). Part of its ancestral (plesiocoric) area is the Iberian Peninsula, where two mitochondrial races of morphologically indistinct rabbit populations in the northeast and southeast have been distinguished, tracing back their divergence to at least 2 Ma (Branco et al. 2000). The recorded age of the species in contrast seems much younger. The oldest fossils attributed to the modern European rabbit species are around 0.5 Ma old (Middle Pleistocene, Lopez Martinez 1989; Donard 1982). Extinct species of European rabbits have been described in western European fossil

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sites (+*Oryctolagus laynensis*, 3.5 Ma; +*Oryctolagus lacosti*, 2 Ma, +*Oryctolagus burgi*, 0.3 Ma). They would allow reconstructing the history and dating the divergence of their recent relative, the European rabbit.

In this work, I present an overview of the lagomorph fossil record as a frame for evolutionary questions concerning this peculiar mammalian order, particularly for the problem of the origin and dispersal of the most widespread recent lagomorph, the European rabbit. Evolutionary biology as any other branch of biology needs paleontological data as a necessary reference for contrasting the results of different approaches (genetic, biochemical, ecological, etc.) and putting the results into their chronological and paleobiogeographical framework.

The Lagomorph Fossil Record

Most of the lagomorph fossil taxa are known mainly by their dentition, because incisors and cheek-teeth are easily preserved and recognized in fossil samples. Fortunately, these are very informative elements also widely used for modern lagomorph taxonomy, making possible the comparison between both types of data. In many cases, maxillae, jaws, and isolated postcranial material (mainly anklebones, girdles, and epiphyses of long limb bones) of fossil lagomorphs have been also identified and described. Skulls and articulated, complete skeletons are sometimes also preserved (Wood 1940; Dawson 1958; Masini 1989), and there are rare cases where soft parts, body outline, and hair are also observable (Mein et al. 1983). Since a less number of characters are available in fossils for taxonomic distinction, the fossil record shows generally a lower resolution for diversity estimates than the biosphere does. The study of lagomorph fossil remains is particularly difficult as Dawson (1967) pointed out, in part because of the conservative body plan and the large intrapopulational variability of this mammalian order.

The lagomorph fossil record contains about 78 genera and 234 species after checking 2742 fossil localities in four continents. I refer extinct taxa by a '+' preceding names. Palaeoenvironmental data have greatly increased and I do not refer here to. The record is now twice larger than in 1967 when Dawson made a first revision but has not substantially changed the main pattern with three groups of taxa: (1) gliroid mixodont mammals relatives of lagomorphs, (2) stem-lagomorphs and (3) lagomorph ascribed to both modern families.

(1) Gliroid mixodont mammals, relatives to both Lagomorpha and Rodentia, are recorded in Asia from the late Paleocene to the Oligocene (58–30 Ma ago);
(2) Stem-lagomorphs, the basal group of primitive lagomorphs, appeared in Asia around late Eocene times (about 45 Ma), spread into North America and Europe and persisted until the late Miocene (about 10 Ma); and,
(3) Living families Ochotonidae and Leporidae differentiated respectively in Asia and North America in Late Eocene–Early Oligocene (around 33–35 Ma ago).

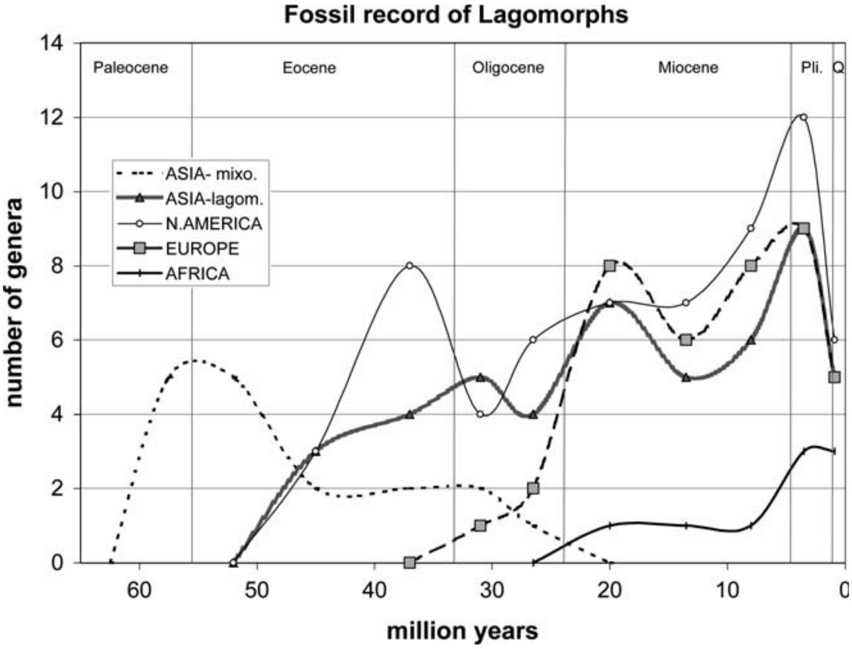

Fig. 1 Pattern of diversity of the lagomorph fossil record and their relatives, the gliroid mixodonts, during the Cenozoic era. Number of genera in four continents is plotted against age at the mid-point of the geological periods

The overall pattern of lagomorph fossil diversity is shown in Fig. 1, with the rise and fall of gliroid mixodonts in Paleocene–Early Eocene times, followed by a rapid spreading of stem-lagomorphs in Asia and North America, replaced later by the modern lagomorph families, which colonized Europe and subsequently, Africa. They underwent an important radiation around Pliocene times (5 Ma ago) and suffered a severe decline in Quaternary times, mostly in the Holartic region (Northern Hemisphere).

Origins of Lagomorphs

Several criteria indicate that Asia is the ancestral area of the order Lagomorpha:

- the oldest unambiguous, true lagomorphs (+*Lushilagus*) occur in China in Middle Eocene (around 45 Ma);
- lagomorphs in general, and stem-lagomorphs in particular, reached their higher diversity in this continent;
- the origin of lagomorphs has been related with the Asiatic mixodonts (order Mixodontia, Sych 1971). This endemic order of mammals bearing

a mosaic of characters of rodents and lagomorph, lived in Asia mainly during late Palaeocene to Oligocene (about 58 to 30 Ma). One of its families (Mimotonidae) has been proposed as the sister group of Lagomorpha (Li and Ting 1985, 1993).

- the order Rodentia, an alleged sister group of Lagomorpha and Mimotonidae, is also related to the mixodont family Eurymylidae and made their earliest appearance in Asia.

Only two families (Mimotonidae and Eurymylidae), 12 genera and about 20 mixodont species are known by now, but their fossil quality, number, and observations are currently rapidly increasing (e.g., Dashzeveg and Russell 1988; Meng et al. 2003; Asher et al. 2005).

The morphological similarities shared by mixodonts, lagomorphs, and rodents lead many authors to include all of them in the Glires superorder. Particularly, small mimotonids (+*Mimotona wana*) share a number of synapomorphies with true lagomorphs (dental roots of upper cheek-teeth within the orbit, peculiar zygoma with external fossa and ventral tubercle, large incisive foramen, grooved anterior upper incisor; Li and Ting 1993). However, the phylogenetic relationships of mixodonts are not stable. Mimotonids cluster either with Lagomorpha (clade Duplicidentata) or with other mixodonts; eurymylids group either with Rodentia (clade Rodentiamorpha) or with Duplicidentata (Averianov 1994; Meng et al. 2003; Asher et al. 2005). The order Rodentia appeared highly differentiated in North America and Asia in late Paleocene, more than 10 million years before the first well-characterized lagomorph.

Some authors interpret the basal lagomorph evolution as a smooth transitional gradation between mixodonts and primitive lagomorphs. However, a direct ancestral-descendent relationship between them is unlikely, because most mixodonts show highly specialized features and larger size in relation to primitive lagomorphs (Sych 1971; Lopez-Martinez 1985; Dashzeveg and Russell 1988; Averianov 1994). Differences concern dental and skeletal features. Primitive true lagomorphs have strongly developed premolars with a labial root, week third molars, open folds in upper premolars, upper molars with a central cusp surrounded by a crescent valley, weak hypocone closely attached to protocone, and narrow and elongated tarsals with a transversal calcaneal canal and a calcaneous-fibular joint. Mimotonids and other mixodonts instead have much reduced or even loss premolars with a closed central basin surrounded by a high cingulum and two labial roots; well-developed third molars much larger than other molars, a central basin in upper molars with strong hypocone well-detached from the protocone and short and widened, primitive tarsal bones. The ratio of third molar length clearly separates both groups (Fig. 2).

True lagomorphs appear in late Middle Eocene, around 15 million years after their late Paleocene closest relative mixodont +*Mimotona* (Fig. 3). A gap exists during the early Eocene between the earliest lagomorphs and their sister group (ghost lineage). Up to now, the taxa from this epoch (+*Aktashmys*, +*Gomphos*) appear less related to lagomorphs than the oldest mimotonids

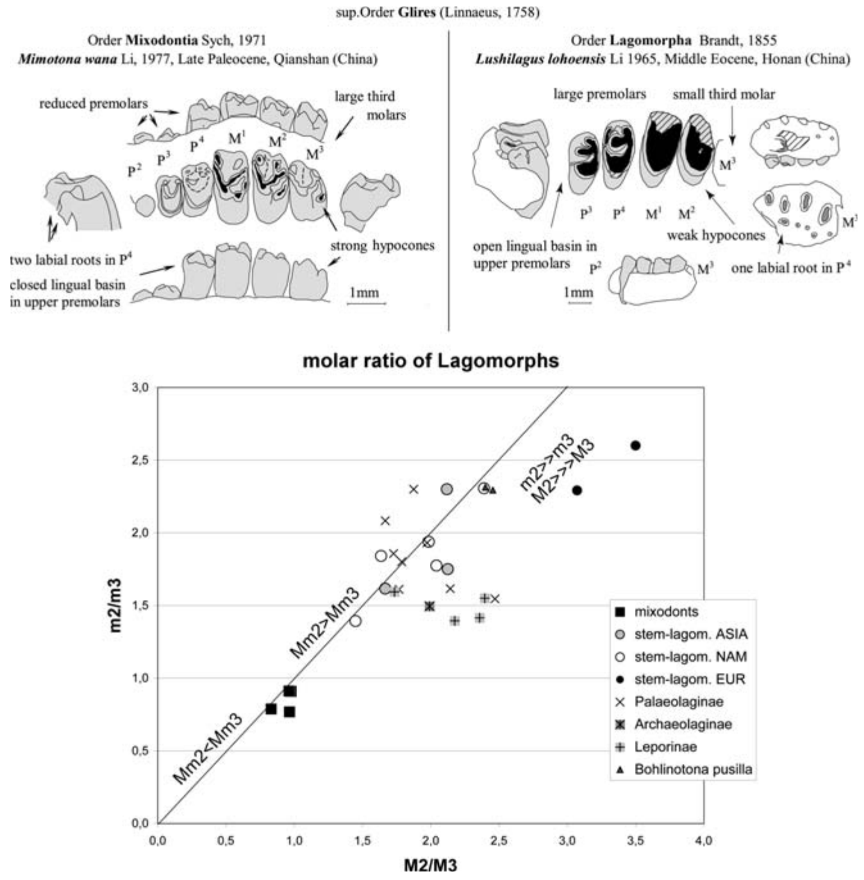

Fig. 2 Distinctive features between mixodonts and true lagomorphs. The scatter diagram represents the length ratio of the third molars against the second molars in mixodonts and different lagomorph groups

from the Palaeocene, and the same occurs with mixodont gliroids from late Eocene and Oligocene (+*Tsaganolagus*, +*Hypsimylus*, +*Mimolagus*). The origin of Lagomorpha may be hidden in this chronological gap (Fig. 3).

The Fossil Record of Stem-Lagomorphs

Traditionally, the systematics of fossil lagomorphs placed all primitive extinct taxa into the extant families Leporidae and Ochotonidae. However, primitive lagomorphs do not share diagnostic, derived characters with the modern families and can be easily distinguished from them. Here we name stem lagomorphs the *plesion* or paraphyletic group of primitive true

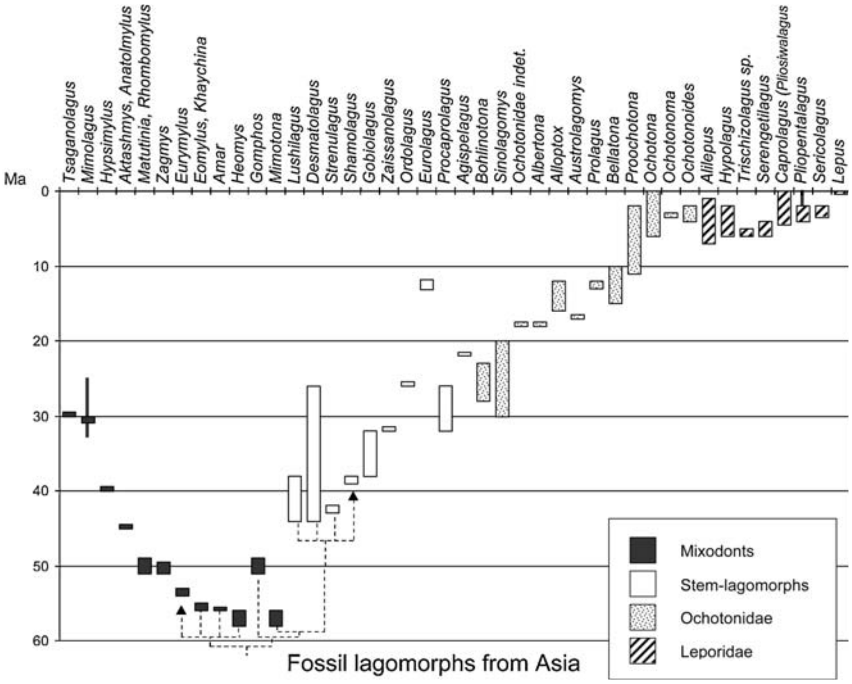

Fig. 3 The fossil record of mixodonts and lagomorphs in Asia. The phylogenetic relationships between both groups is indicated at the base of the chronobiogram, revealing a gap of 15 million years (ghost lineage) between the first lagomorphs and their closest mixodont relative, *Mimotona*

lagomorphs (+*Lushilagus,* +*Shamolagus,* +*Gobiolagus,* +*Procaprolagus,* +*Mytonolagus,* +*Megalagus,* +*Eurolagus,* +*Gripholagomys*) included by some authors in the families 'Palaeolagidae' and 'Amphilagidae'.

Stem lagomorphs have relatively low-crowned rooted cheek-teeth with a complicated dental pattern bearing persistent folds and cusps. In the upper cheek-teeth, the arrangement of cusps shows a peculiar central one (whose homology with that of other mammals has been extensively debated), surrounded by a crescentic, curved valley. The upper cheek-teeth are highly asymmetric; their crowns are higher in the lingual side and curves laterally as the lingual root grows more than the labial ones (see Fig. 4). A tubercle in the maxillary bone containing the dental roots enters the orbit. The long-lasting milk teeth are large. The third molars are highly reduced compared to mixodonts, but relatively large compared to Leporidae or Ochotonidae. The lower cheek-teeth show a conspicuous third lobe (hypoconulid), which disappears later with wearing. Their highly characteristic first lower premolar has a small, narrow trigonid and lacks an anteroconid. The skull and skeletal features show highly derived, diagnostic states (in zygoma, orbits, bony palate, vertebrae, tibia and tarsals; see Lopez-Martinez 1985). The size is generally small; exceptionally, some of them reached a large size (+*Megalagus*, +*Gymnesicolagus*).

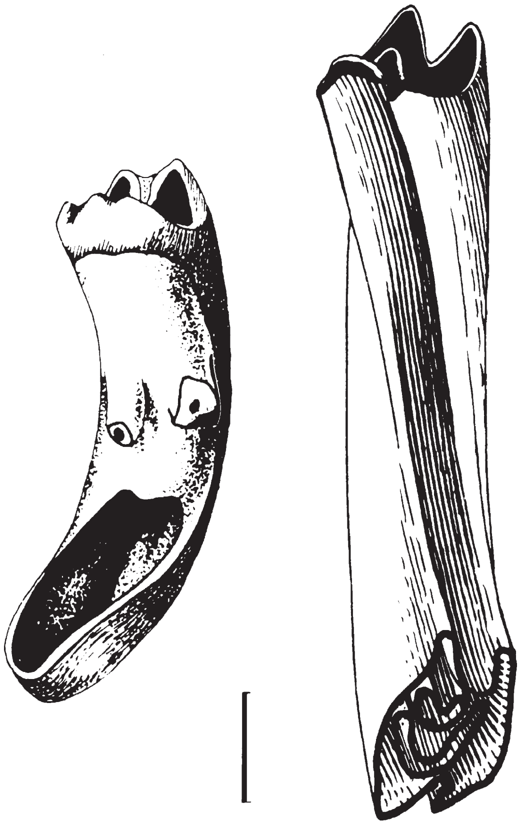

Fig. 4 Hypsodonty in upper cheek-teeth of stem-lagomorphs (*left*) compared to fossil lagomorphs related to the modern family Ochotonidae (*right*)

Well-characterized stem lagomorphs first appeared in Asia (+*Lushilagus*, +*Strenulagus*, +*Shamolagus*, +*Gobiolagus*, +*Desmatolagus*, etc.) in Middle Eocene (about 45 Ma ago) and afterwards in North America (+*Mytonolagus*, +*Tachylagus*, +*Procaprolagus*) in the late Eocene (about 43 Ma, Fig. 5). By the latest Eocene and early Oligocene (36–30 Ma), the group shows a rapid diversification (+*Megalagus*, +*Desmatolagus*, +*Ordolagus*, +*Agispelagus*, +*Zaissanolagus*) and try the invasion of Europe with low success (+"*Shamolagus*" *franconicus*, +*Desmatolagus* sp.). Only at the end of the Oligocene (about 25 Ma ago) a new wave of immigrants successfully colonizes Europe (+*Amphilagus*, +*Piezodus*), already differentiated as primitive ochotonids.

Some stem lagomorphs persisted after the appearance of well-differentiated leporids and ochotonids in Early Oligocene, a golden epoch of lagomorph diversity where up to eight lagomorph taxa in Asia and four in North America are recorded in sympatry. During the Miocene they spread into the Northern Hemisphere (+*Megalagus*, +*Gripholagomys*, +*Russellagus*, +?*Desmatolagus*, +*Eurolagus*, +"*Amphilagus*"...) and became completely extinct by late Miocene

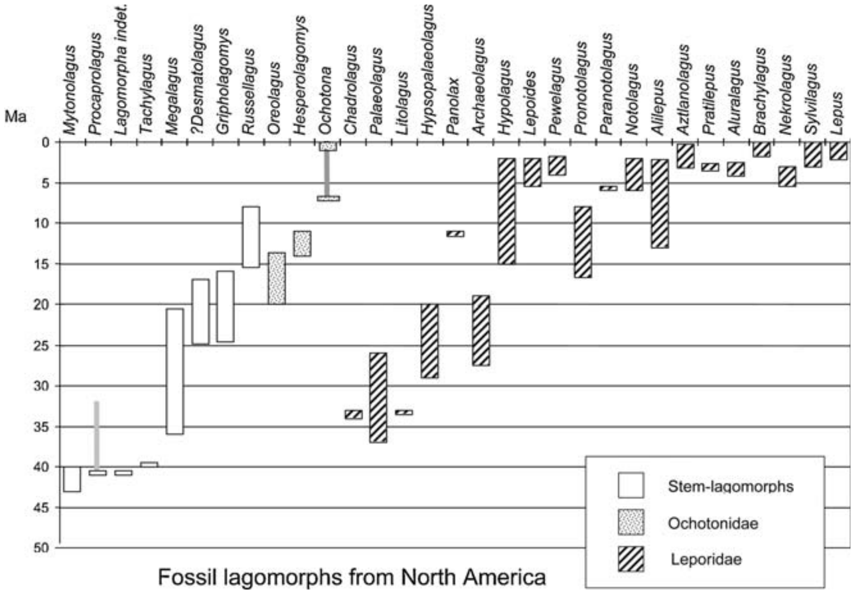

Fig. 5 Chronobiogram of the lagomorph fossil record in North America

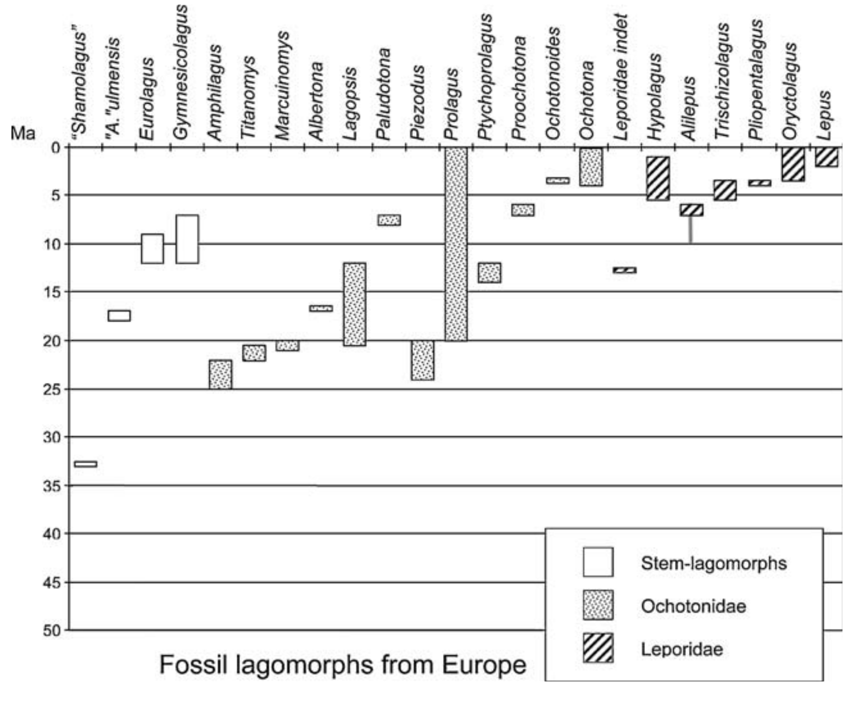

Fig. 6 Chronobiogram of the lagomorph fossil record in Europe

times. One of the largest and latest surviving stem lagomorphs was the highly modified +*Gymnesicolagus*, extinct about 7 Ma ago in the Balearic Islands (Fig. 6).

Many authors include the stem lagomorphs in the family Leporidae because they share a primitive dental formula (2/1 i, 0/0 c, 3/2 p and 3/3 m). However, this is a primitive feature of the order, and natural groups or clades must instead be defined by shared derived features. Thus stem-lagomorphs are an outgroup for Ochotonidae and Leporidae (McKenna 1982). Primitive members of the modern lagomorph families have common derived features not shared by stem lagomorphs, such as rootless adult teeth, loss of the crescentic valleys in early wear stages, permanent hypostriae that do not close up with wear, and simplified, very reduced third molars. The origin of the lagomorph modern families is related to the stem-lagomorph genus +*Desmatolagus*, recorded in early Oligocene (33 Ma) of northern continents and attributed either to Ochotonidae or Leporidae by authors. Some species of this genus are closer to the Asiatic Oligocene Ochotonidae (+*Bohlinotona,* +*Sinolagomys*), but the first true leporids appeared and spread in North America (+*Palaeolagus,* +*Chadrolagus*), phylogenetically related to the stem-lagomorph +*Mytonolagus* (Gawne 1978). Therefore, the derived features shared by both modern lagomorph families were probably independently attained in each continent from different ancestors.

The Fossil Record of ochotonids

The way ochotonids obtained the total hypsodonty of the upper cheek-teeth is different from that of the leporids, which strength the hypothesis of an independent origin of both families. Ochotonids produce continuous-growing cheek-teeth by a strong torsion of the shafts, rejecting the buccal roots and cusps towards the exterior of the maxilla (Fig. 4). The curved labial roots of the upper cheek-teeth are contained in a convex maxillary tubercle connected to the zygoma. They lose the upper third molar (M3) and reduce the lower third molar to a single lobe. Trigonids and talonids are permanently isolated. The palate shows a conspicuous premolar foramen. These features are probably derived, while the non-molarized upper premolars, the basicranial horizontal profile and the presence of two mental foramina in the jaw are probably primitive features.

Ochotonids first appeared in central Asia during the Oligocene (+*Sinolagomys,* +*Bohlinotona*). Their origin is close to the small-sized species of +*Desmatolagus*. At the end of this period they spread to Europe (25 Ma ago, Fig. 6), where a local radiation of tropical ochotonids swarmed until recent times (+*Titanomys,* +*Marcuinomys,* +*Albertona,* +*Lagopsis,* +*Piezodus,* +*Prolagus*, etc.). Ochotonids entered North America (+*Oreolagus,* +*Hesperolagomys*), Africa (+*Austrolagomys*) and India (Ochotonidae indet.) by the Early Miocene, around 18 Ma ago (Fig. 7), while +*Alloptox* and

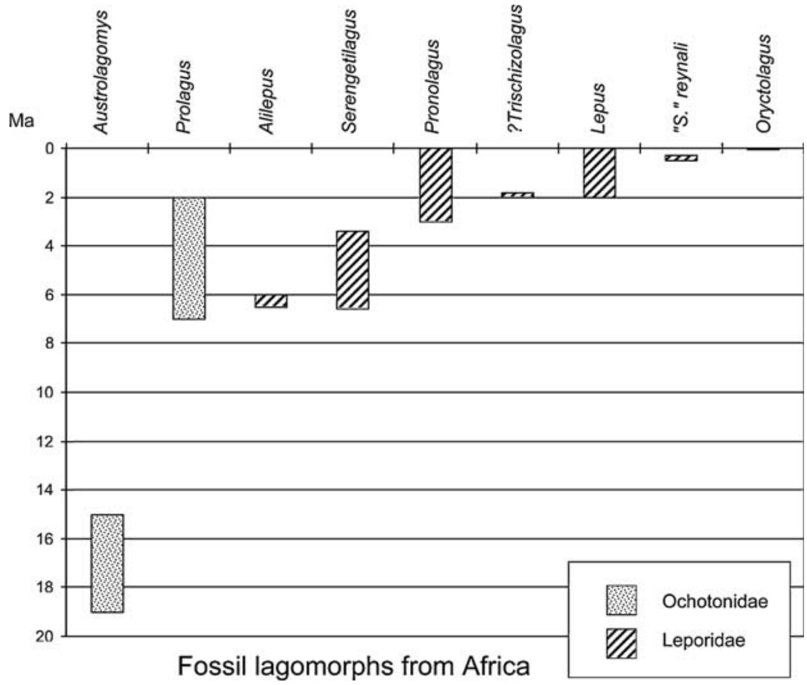

Fig. 7 Chronobiogram of the lagomorph fossil record in Africa

+*Bellatona* replaced the primitive Ochotonids in Asia during the Miocene. Most of them were extinct by the late Miocene, the only survivor being +*Prolagus* in Europe (Russian authors consider it a different family, 'Prolagidae'). It entered in NW Africa at the end of Miocene (6 Ma ago), but finally its last species (+*P. sardus*) became extinct in Corsica and Sardinia few hundreds years ago.

A new wave of Ochotonids (+*Proochotona*, +*Ochotonoides*, +*Ochotonoma* and *Ochotona*) formed in Asia in Mio-Pliocene and spread in Northern Hemisphere where *Ochotona* persist today, except in Europe where they became extinct. The fossil record of the modern genus *Ochotona* documents many extinct Pliocene and Pleistocene species: about 12 species disappeared in Asia (e.g., +*Ochotona lagreli*, +*Ochotona antiqua*, +*Ochotona transcaucasica*, +*Ochotona niewanica* and +*Ochotona tologolica* among others), three in Europe (+*Ochotona polonica*, +*Ochotona valerotae* and +*Ochotona horaceki*) and two in North America (+*Ochotona spanglei* and +*Ochotona whartoni*). In contrast, most of the living *Ochotona* species (around 22–26) have a poor fossil record restricted to the last few thousand years (*Ochotona erythrotis*, *Ochotona princeps*, *Ochotona pusilla*, *Ochotona thibetana*). Thus, the diversity turnover of modern pikas seems relatively as high as in other quaternary specious micromammals like arvicolid rodents although lagomorphs are poorer than rodents in diversity and abundance, either in the fossil record or the biosphere.

The Fossil Record of leporids

In contrast to ochotonids, leporids obtained the total hypsodonty of the cheek-teeth by straightening the crown shafts. Their roots locate in a vertical tubercle entering the orbit separated from the zygoma. Upper premolars are molarized and the upper third molar (M3) is preserved but reduced to a single lobe. Trigonids and talonids have lingual connections and the mandible has a single mental foramen. The skull has a basicranial angle of near 30°. These features are probably derived, while the presence of a bilobed lower third molar is a primitive feature. Other apparently primitive traits, such as the premolar foramen extremely reduced or absent and the uniserial incisor enamel could in fact be secondary acquisitions.

The family Leporidae is divided into three subfamilies: Palaeolaginae, Archaeolaginae and Leporinae according to the lower p3 pattern (Dice 1929; Wood 1940). Palaeolaginae p3 is hour-glass-shaped (trigonid and talonid connected by a central narrow isthmus). Archaeolaginae p3 has trigonid and talonid connected by a large lingual isthmus, which is the primitive pattern for most stem lagomorphs (Fig. 8). Leporinae p3 has trigonid and talonid

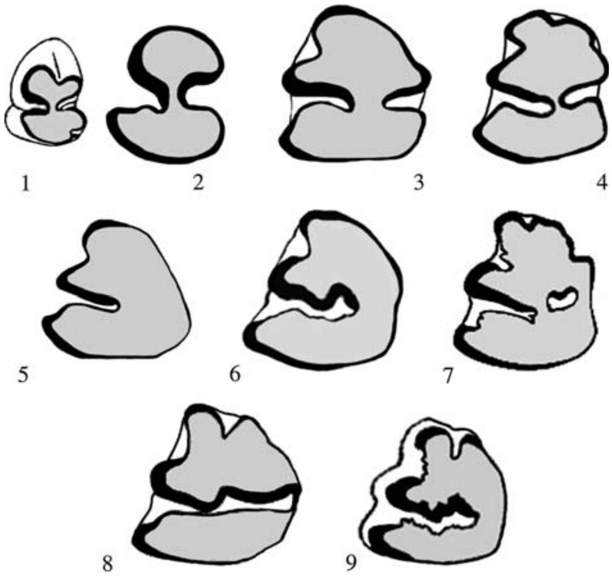

Fig. 8 Pattern of the leporid p3, one of the most distinctive morphological features of lagomorphs. *Upper row* palaeolagine-types. *Middle row* archaeolagine-types. *Lower row* leporine-types. Transitions are observed between palaeolagine and archaeolagine types and between archeolagine +*Nekrolagus* or +*Aluralagus* types and leporine type, but not between palaeolagine and leporine types *1* young +*Palaeolagus* (+*P.haydeni*). *2* adult +*Palaeolagus* (+*P. haydeni*). *3* +*Alilepus* (+*A. annectens*). *4* +*Trischizolagus* (+*T. dumitrescuae*). *5* +*Hypolagus*. *6* +*Aluralagus*. *7* +*Nekrolagus*. *8 Lepus*. *9 Sylvilagus* (+*S. hibbardi*). Redrawn from Wood (1940), White (1991) and different works by Daxner, Fejfar, Qiu, Radulescu and Sampson

connected by a narrow lingual isthmus like other lower cheek-teeth. Based on current phylogenetic hypotheses, authors include some Palaeolaginae in the Leporinae subfamily (Dawson 1958; White 1991; Voorhies and Timperley 1997), but as a consequence systematics became unstable and lacks subfamilial diagnostic characters.

The first Palaeolaginae leporids (+*Paleolagus*, +*Chadrolagus,* +*Litolagus,* +*Hypsopalaeolagus*) related to the stem lagomorph +*Mytonolagus* appeared and diversified in the latest Eocene (38 Ma ago) in North America, the homeland of the Leporidae (Fig. 5). Palaeolaginae are particularly abundant in the Western Interior during the Oligocene, when they attain the molarization of P3-P4 and add an anteroconid to p3. Later on, some Palaeolaginae leporids (+*Panolax*) coexisted with their sister group Archaeolaginae (+*Archaeolagus,* +*Hypolagus,* +*Lepoides,* +*Pewelagus*) documented from Miocene to Pleistocene (23–0.3 Ma). Archaeolaginae already loss almost all primitive cusps and valleys in early stages of tooth wear, and their p3 adds an anteroexternal fold. +*Hypolagus* is the most prolific and widespread archeolagine in North America, where 12 species are known from Middle Miocene to Pliocene (15–2 Ma). It spreads in Eurasia (5 species in Asia, 3 species in Europe) in latest Miocene and subsisted in Sicily Island until the Pleistocene. Rare leporid findings are recorded in Europe since Middle Miocene (about 13 Ma ago), but they do not settle definitely there until the latest Miocene, a case similar to the first European lagomorph invasion.

The history of Leporinae can be traced back to their putative common ancestor +*Alilepus*, known in North America from late Miocene to late Pliocene (10–2 Ma) and spreading in the Old World around 7 Ma ago where subsisted until Early Pleistocene. Its origin has been related to the genus +*Pronotolagus* from middle to upper Miocene (16–8 Ma), characterized by a four-folded p3 probably derived from primitive Archaeolaginae or advanced Palaeolaginae. Both +*Pronotolagus* and +*Alilepus* show a palaeolagine-type p3 but have been classified as Leporinae based on their phylogenetic relationships with their successors. Between 16 and 6 Ma there are few fossils attributed to Leporinae (Voorhies and Timperley 1997). In contrast, a remarkable diversity burst explodes in the Pliocene, when more than 16 new leporid genera appeared. Among them are the Leporinae +*Aluralagus*, +*Aztlanolagus*, +*Nekrolagus,* +*Notolagus*, +*Paranotolagus*, +*Pratilepus,* +*Trischizolagus* (Figs. 3, 5, 6 and 7), and the largest lagomorph, a giant +*Alilepus* relative recorded from Minorca Island. Local diversity is also remarkable; up to seven coexisting lagomorph taxa are cited in a single Pliocene local assemblage from North America. All these Pliocene leporids became extinct by late Pleistocene or Holocene times.

Living leporids with a palaeolagine-type p3, which appear as a natural group in some molecular phylogenies, are poorly represented in the fossil record. Among them, the Japanese *Pentalagus* is the only one with a fossil relative, +*Pliopentalagus* from the European and Asiatic Pliocene. It assesses the refugee status of the surviving insular Amami rabbit. From the remaining palaeolagine-like living taxa (*Bunolagus, Pronolagus,* and *Romerolagus*), only *Pronolagus* has been documented by fossil remains from South African Plio-Pleistocene.

Most living leporids (*Oryctolagus*, *Sylvilagus*, *Caprolagus*, *Lepus*) present the leporine p3 pattern. No transitional populations are known whose p3 transformed from palaeolagine to leporine type. In contrast, two morphologically intermediate p3 types exist (Fig. 8): +*Nekrolagus*-type p3, with an enamel lake between two isthmus connecting trigonid and talonid (present in rare *Oryctolagus* and *Lepus* specimens); and +*Aluralagus*-type p3, with a rather wide single lingual isthmus (sometimes observed in *Sylvilagus*). This would confirm phylogenetic proposals suggesting an independent acquisition of the leporine p3 pattern through parallel evolution across a cladogenetic radiation (Dawson 1958; White 1991; Averianov 1999).

Among living Leporinae, *Oryctolagus* has the oldest fossil record of around 3.5 Ma (see below). Other modern Leporinae genera appeared one-half to 1 million years later (*Sylvilagus* 3 Ma ago and *Lepus* 2.5 Ma ago in North America; *Caprolagus* 3? to 1.8 Ma ago in India and Pakistan). *Nesolagus* and *Poelagus* lack fossil references. Africa and SW Asia have a poorly studied fossil record that may probably change this picture.

Like living Ochotonidae, living Leporinae are represented in the fossil record by extinct species: +*Brachylagus coloradoensis*, +*Caprolagus lapis,* +*Caprolagus (Pliosiwalagus) sivalensis*, +*O. laynensis* (Fig. 9); +*O. lacosti,* +*O. burgi*, +*Sylvilagus hibbardi*, +*Lepus terraerrubrae*, +*Lepus veter*, +*Lepus*

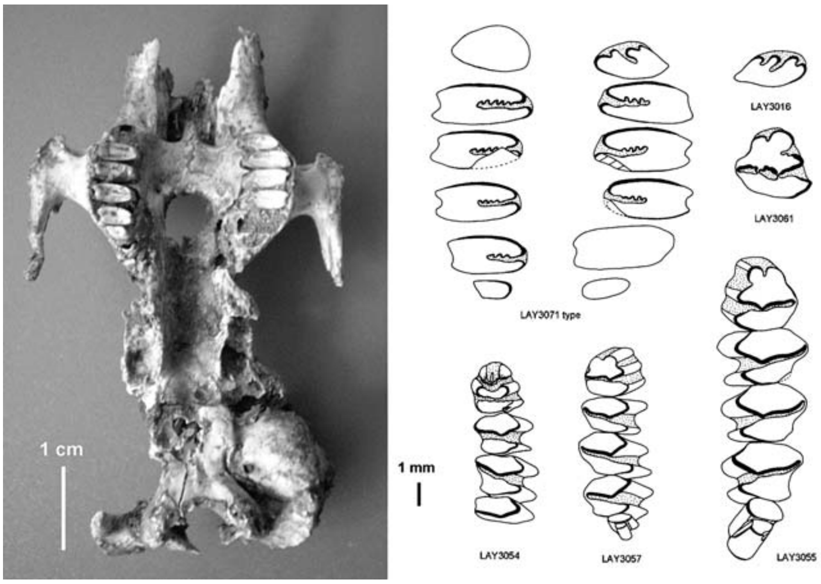

Fig. 9 +*Oryctolagus laynensis*. Ventral view of the skull and occlusal views of the dental rows. Middle Pliocene (3.5 Ma), Layna (Soria, central Spain) (from López-Martínez, 1977, 1989)

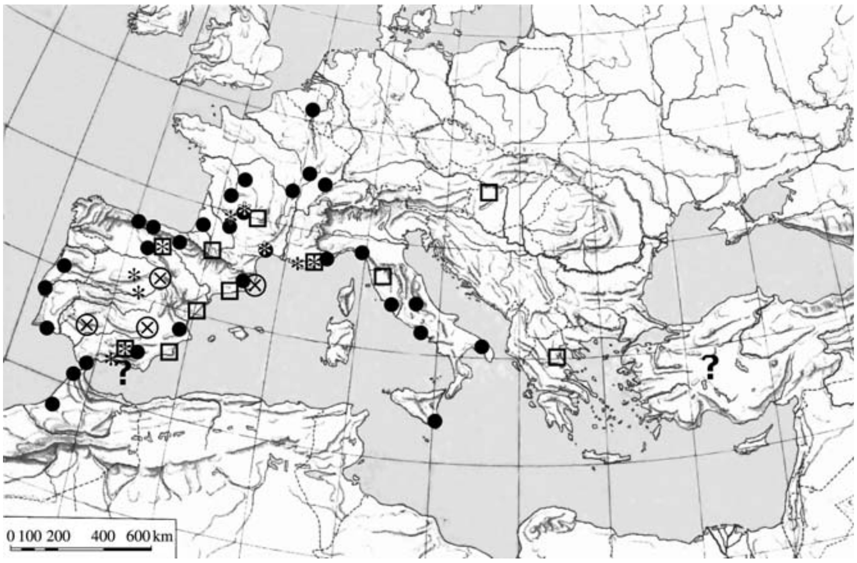

Fig. 10 Map of *Oryctolagus* fossil findings. *Crossed circles +O. laynensis*, Middle-Late Pliocene (3.5–2 Ma). *Squares +O. lacosti* and *O.* sp, Late Pliocene–Early Pleistocene (2.5–0.8 Ma). *Stars +O. lacosti*, *O. cuniculus* and *+O. burgi*, Middle Pleistocene (0.8–0.1 Ma). *Dots O. cuniculus*, Upper Pleistocene (0.1–0.01 Ma)

tanaiticus, etc. In some cases, fossil attributions to modern species are well supported by morphological data (*Lepus* cf. *californicus* and *Sylvilagus* cf. *audubonii* in late Pleistocene of North America, *Lepus cf. granatensis* in middle Pleistocene of Spain, *Lepus timidus* in late Pleistocene of Switzerland, France and Euskadi in Spain). Other cases have not yet been justified enough (*O. cuniculus* in Pleistocene of Anatolia and Africa, Fig. 10; *Lepus europaeus* in France; *L. timidus* in the central Iberian Peninsula).

The Fossil Record of the European Rabbit

The European rabbit genus *Oryctolagus*, now spread worldwide, appeared in the fossil record before any other modern Leporinae genus. The first recorded *Oryctolagus* species is *+O. laynensis* from Middle Pliocene (about 3.5 Ma) from northeastern, central and southern Spain and probably southeast France, associated to an arid, warm (sub)tropical savannah-type fauna. Earlier indices from a late Miocene site in southern Spain may be distrusted. This site with equatorial African mammals dated to 6 Ma has delivered an isolated P2 tooth tentatively attributed to *Oryctolagus*, yet its morphology may agree with advanced *+Trischizolagus* species recorded from Spanish

Pliocene sites. Albeit previous conjectures of an African origin, the European rabbit has not been confidently signaled in Africa during late Miocene to Pleistocene times (8–0.1 Ma ago), when species attributed to the leporid genus +*Serengetilagus* dominates most assemblages from northern, central and eastern Africa (Fig. 7). Thus the fossil data indicates the Iberian Peninsula as the probable ancestral area of the European rabbit.

If the biogeographical origin of *Oryctolagus* seems to be somewhat constrained, its phylogenetic origin is less clear. The widespread Leporinae +*Alilepus* and after +*Trischizolagus* were precursors of *Oryctolagus* in its ancestral area and its surroundings. +*Trischizolagus* has been proposed as a probable *Oryctolagus* ancestor, as well as for *Lepus* and other leporines, although no transitional populations have yet been found. Instead, cladistic phylogeny links *Oryctolagus* more closely to *Lepus* than to *Trischizolagus* (Averianov 1999). Molecular phylogenies give also contradictory results, connecting *Oryctolagus* either to *Lepus*, to *Bunolagus* or to *Caprolagus* (Halanych and Robinson 1999; Mathee et al. 2004).

During the Mio-Pliocene transition (6–3 Ma), the leporids +*Alilepus,* +*Hypolagus,* +*Trischizolagus*, +*Pliopentalagus* and +?*Serengetilagus* were present in Europe; but none of them was associated with the oldest *Oryctolagus* species, +*O. laynensis*. Up to now, this ancient rabbit has only been recorded from 3.5–2.5 Ma in Spain (sites in Castilla-Leon, Castilla-La Mancha, Catalonia and Western Andalousie) and probably in southeast France. The Iberian +*Trischizolagus* (sites in Aragon, Valencia, Murcia and eastern Andalousie from 6–3 Ma) preceded and clearly overlapped its chronological distribution with *Oryctolagus*, but they did not occur in the same localities. Iberian +*Trischizolagus* record the most primitive species of the genus (+*Trischizolagus crusafonti* from Late Miocene and +*Trischizolagus maritsae* from early Pliocene), both different and small-sized that the type species +*Trischizolagus dumitrescuae* from the Late Pliocene of Eastern Europe. *Lepus* appears as a late immigrant, first recorded around 2 Ma ago in Eastern Europe, and about at the same date in North America (not much older than 2.5 Ma). It could be older in Africa. Thus, the fossil record suggests that *Oryctolagus* and *Lepus* do not share a common ancestral area, although both may be related to their predecessors +*Alilepus* and +*Trischizolagus*.

+*O. laynensis* was a robust rabbit with relatively wide choanae, long bony palate, small auditory bullae, large posterior molars, shallow hypoflexus, relatively short, asymmetric anteroconids and a frequent enamel lake in the p3 (Fig. 9). No transitional forms are known between this species and the modern *O. cuniculus*. However, they appear closer to each other than +*O. lacosti*, a larger-sized rabbit species recorded between 2.5 and 0.6 Ma in southwest Europe (Spain, France, Italy and isolated findings from Hungary and Greece). The distinctive features of this peculiar rabbit (deep crenulated hypoflexus, short palatine portion of bony palate, choanae width larger than palatal length, large, robust skeleton) mislead some authors to describe it as a hare ('*Lepus valdarnensis*', '*Lepus etruscus*', '*Lepus issiodorensis*'. . .). Its limb ratio and p3 morphology (large lingual anteroconid, longitudinally oriented anterior fold) are however clearly rabbit-like. Another large rabbit species (+*O. burgi*) possibly related to +*O. lacosti* has been described in Middle Pleistocene of Italy.

The living species *O. cuniculus* is first recorded in southern Spain in Middle Pleistocene (about 0.6 Ma) associated to a relict, warm-type fauna. An Early Pleistocene reference to this species in Turkey needs to be confirmed. Relatively robust races of this species are recorded in Middle Pleistocene of France and the central Iberian Peninsula (+'*O. c. lunelensis*', +'*O. c. grenalensis*'), contemporaneous of the large-sized lineage +*O. lacosti* -+*O. burgi*. In the Late Pleistocene, only the modern rabbit survived, spreading to peri-Mediterranean area and northern Europe where it coexisted with *L. timidus* (Fig. 10). During the maximum glacial period and Early Holocene, the European rabbit seems again confined to the Iberian Peninsula and southern France, where it constituted an important trophic resource for the survival of large predators. The European rabbit suffered a remarkable genetic differentiation in Iberia during the glacial period (Branco et al. 2002; Callou 2003). Its size was then larger than the recent Iberian rabbits, and increased again, reaching 15% more length during the Neolithic period, until its worldwide anthropic expansion.

Remarks and Discussion

The fossil record is a unique way of documenting the past history of an organism and testing our hypotheses about their evolution. Initiatives such as 'The Palaeobiology Database' implemented by J. Alroy greatly contribute to this goal. Concerning lagomorphs, important advances in phylogenetic studies in exploration of Asiatic fossil localities and a two-fold increase in the number of fossil lagomorph taxa can be noted in the later years. Even so, through the numerous studies and materials collected by different approaches, techniques and authors, the timing and diversity pattern of the lagomorph succession shown by the fossil record have a remarkable stability, which allows us to assess its reliability. Through the joint efforts of palaeontologists, the fossil record of lagomorphs, continuously refined and improved, shows confidently a solid pattern, in spite of minor discrepancies in systematic distinction and in the nomenclatural translation of phylogenies.

The pattern of the lagomorph fossil record can be compared with phylogenetic and phylogeographic trees of extant lagomorph evolution proposed with molecular data. Apart from discrepancies between morphological- and molecular-based phylogenies, which are common to many other cases, disagreements appear when comparing the ages of divergence for lagomorph sister taxa inferred by the molecular clock and by the fossil record.

The origin of Lagomorpha according to the fossil record is between 60 Ma (ghost lineage -'long fuse' model), and 45 Ma (stem lagomorph 'explosive' model) (Fig. 3), while for molecular clock is 71 Ma (estimates ranging between 81 and 28 Ma). The fossil constraint for dating the divergence between ochotonids and leporids (20–40 Ma) is at odds with molecular studies indicating 50 Ma age. According to the molecular clock, all modern Leporidae genera are Miocene in age (14–8 Ma), against a Pliocene age (4–2 Ma) according to the fossil record. Another important disagreement is the *Lepus* origin, dated by molecular phylogeny between 4 and 11.8 Ma (much earlier than rabbits according to Matthee et al. 2004), while the opposite is verified in the fossil record (*Lepus* appearing 2.5 Ma ago, later than rabbits, around 4 Ma).

In front of the numerous disagreements in this and many other cases, some authors discard the morphological data because of convergences, and the fossil data classically qualified as 'incomplete' (which ironically supposes that other data sets are more 'complete'). In fact, many cases show a high level of coincidence between morphological, paleontological, and molecular studies. Poor resolution and disagreement problems are often attributed to rapid evolutionary radiations. The fossil record is rich in examples of explosive evolution, like lagomorphs showing a nearly complete renewal of genera and species in a very short time. The 'explosive' model of evolution is now admitted as data, not noise, and is even observed in fossils of very recent age, when maximum chronological resolution can be obtained.

Conclusions

Fossil lagomorph taxa, which doubled in the last 40 years, show a general stability in chronology and diversity pattern. The main results of this survey are summarized as follows:

(1) The lagomorph fossil record contains about 78 genera and 236 species in Old World and North America. The number of living taxa is much lesser, indicating a decline in the evolution of this mammalian order. The number of coexisting species has also decreased. Nowadays, more than three sympatric lagomorph species is extremely rare, while up to eight sympatric lagomorph species are recorded in Asia in Paleogene times and up to seven in North America in Neogene.

(2) Around 60 Ma is a maximum age for the divergence between lagomorph and mixodont Glires, implying a ≈15-Ma gap (ghost lineage) between the first true stem lagomorphs and their mixodont sister group *Mimotona*. Alternatively, lagomorphs could appear later on (around 45 Ma) by explosive radiation.

(3) Asia is probably the ancestral area for Lagomorpha, as well as for Ochotonidae, first recorded there about 33 Ma ago.

(4) North America is the ancestral area of Leporidae (family definition restricted to advanced lagomorphs, excluding stem lagomorphs). The first Leporidae appeared there about 37 Ma ago. Leporidae and Ochotonidae thus evolved independently from different stem lagomorphs in different areas.

(5) Modern Leporinae are related to +*Pronotolagus* and +*Alilepus*, first recorded in North America between 16 and 13 Ma ago.

(6) Important time lags between first arrival and definite settlement are observed: e.g., in Europe a 8-Ma lag time occurred between lagomorph entry (33 Ma ago) and final settlement (25 Ma ago). Also, 6 Ma elapsed for Leporidae settlement in Europe (recorded 13 Ma ago but extremely rare until around 7 Ma ago), and for *Ochotona* in North America (documented around 6 Ma ago, then absent until a new arrival 2 Ma ago).

(7) Explosive radiation occurred in Pliocene times, when more than 16 new lagomorph genera appeared in the four continents. Most of them became extinct at the end of this period or during the Quaternary.

(8) Many living lagomorph genera lack a fossil record. The others are mainly recorded by extinct species, indicating a recent renewal of the lagomorph fauna.

(9) The most widespread lagomorph, *Lepus*, is poorly represented in the fossil record. It is confidently recorded around 2.5 Ma ago, later than several rabbit genera.

(10) The European rabbit has one of the longest leporid fossil records, dating back to 3.5 Ma years ago in the Iberian Peninsula. One of its precursors, +*Alilepus* or +*Trischizolagus*, could be its ancestor. Four parapatric species from two lineages spread in Western Europe. The living rabbit, recorded around 0.6 Ma ago in southern Spain, is the only survivor.

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Inferring the Evolutionary History of the European Rabbit (*Oryctolagus cuniculus*) from Molecular Markers

Nuno Ferrand

Introduction

The European rabbit (*Oryctolagus cuniculus*) is a well-known species all over the world and occurs in both wild and domestic forms. European rabbits are present in most of Europe, North Africa, parts of South America, Australia and New Zealand, as well as in more than 800 islands, where they occupy a huge variety of ecosystems (Thompson and King 1994). Its domestic counterpart exhibits a remarkable variety of breeds showing variation in colour, size and fertility. Domestic breeds are kept and raised globally for meat, wool and fur, and also as increasing popular pets. However, for most of its history, the European rabbit was confined to the Iberian Peninsula where the species is supposed to have emerged in the mid-Pleistocene. We know this from the analysis of the fossil record that in addition, places also in the Iberian Peninsula the emergence of genus *Oryctolagus* (Lopez-Martinez 2008, this book). The subsequent geographical expansion and successful colonization of multiple territories is as recent as historical times and has been mostly human-mediated. This expansion eventually ended in a domestication process that is unique to Western Europe.

While the fossil record together with much more recent historical documents attesting the origin and recent expansion of the European rabbit provide us with a rough picture of the history of the species, the fact is that in both cases they are represented by scarce and spotty information. This in turn prevents a deeper understanding of the rich and unique evolutionary history of the rabbit species. An alternative way to address questions related to this subject is the analysis of genetic data. In fact, the history of species, including their split from sister taxa, historical population subdivisions, expansions and contractions, and other types of demographic events, leave a signature in their genomes that can be assessed by using a combination of molecular biology tools and statistical inference procedures. This is particularly true in the last few years, which witnessed the dramatic development

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of different types of molecular markers able to provide different, but complementary, information on the evolutionary trajectories of species (Avise 2004). In a mammalian species genetic information is typically packed in four different genomic compartments, which different properties will be briefly discussed below and exemplified in the subsequent sections.

The Different Genomic Compartments of a Mammal

The four different genomic compartments of a mammal are represented in Fig. 1. Most of the genetic information is in the autosomes, in which two genes per sex occur. These genes are bi-parentally inherited and can recombine freely at a rate that varies depending on different genomic features. Different from the autosomes is the peculiar X-chromosome that exhibits two copies in females but only one in males, where it is haploid. As a consequence, while

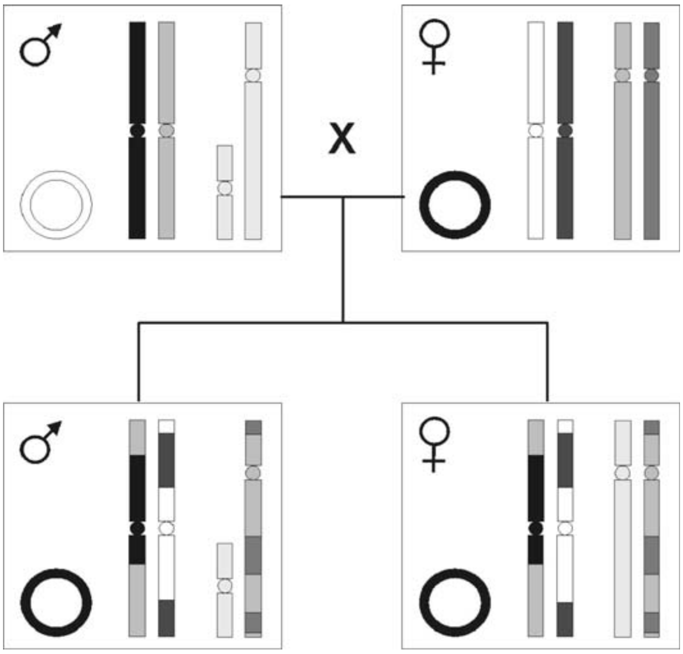

Fig. 1 The four genomic compartments of a mammal. In this two-generation genealogy, the *small circle* represents the mtDNA molecule that is exclusively inherited through the female lineage in spite of being also present in males. The male counterpart is represented by the small Y-chromosome, which occurs only in this sex and defines paternal lineages. In both cases, recombination is absent. The chromosome pair on the left is an example of an autosome, which has two copies in each sex and can recombine in every generation. Finally, the chromosome pair on the right is the peculiar X-chromosome, which has one copy in males and two copies in females, where they are allowed to recombine

being transmitted by both sexes, they pass two-thirds of their time in females, where they can recombine. Finally, the other two genomic compartments correspond to the mtDNA and the Y-chromosome, and both are (1) effectively haploid, (2) uni-parentally transmitted, and (3) have no recombination. The Y-chromosome is exclusive of males and defines paternal lineages. On the other hand, the mtDNA molecule occurs in both males and females, but is exclusively transmitted by females, thus defining maternal lineages.

These different properties have immediate consequences on the type of information that can be obtained from markers residing on these four genomic compartments. In first place, their relative effective population size (N_e) differs and can be viewed, in each mating, as a 4:3:1:1 ratio for the autosomes, the X-chromosome and the mtDNA and the Y-chromosome, respectively. From the coalescent theory, we know that this fact implicates that the time to the most recent common ancestor (TMRCA) will be deeper for the autosomes and the X-chromosome, and shallower for the mtDNA and the Y-chromosome. In principle, this means that using the markers from the first two types of genomic compartments will offer us access to information that is no longer present in the haploid regions. Another relevant aspect of these genomic compartments is related with recombination. In fact, its absence from the haploid compartments of the genome effectively makes them correspond to a single locus, which means that multiple molecular markers from the mtDNA molecule or the Y-chromosome will share the same genealogical history. On the contrary, markers on the autosomes or on the X-chromosome will have essentially independent genealogical histories due to the occurrence of recombination. Today, it is clear that single-locus analyses provide very limited perspectives on the evolutionary history of species, and recent statistical developments are paving the way to the progressive emergence of studies based on the simultaneous use of multiple independent loci (Hey and Nielsen 2004).

Genetic Data and the Evolution of the European Rabbit

Genetic data have been used to address many questions related with rabbit biology, including the analysis of its evolutionary history. This type of data is especially useful when contradictory interpretations exist in the literature based on other evidence, like fossil data. For example, rabbits have been described as autochthonous from Northern Africa by some authors (e.g. Surridge et al. 1999), while others considered them as the result of a recent introduction, probably originated from the Iberian Peninsula (e.g. Dobson, 1998). Along the same lines, fossils are not able to clarify if rabbits persisted since the beginning of the Pleistocene in southern France or if they arrived recently after the last glaciation. In general, the present availability of molecular markers as different as allozymes, microsatellites and DNA sequences allow us to address a suite of questions that are highly relevant for our better understanding of the evolution of this fascinating species.

In this review, I will address five main questions that are of general interest for Lagomorph biologists and students of evolution and will explain how genetic data can be useful to answer them. The first two are related with the distribution of genetic diversity between rabbit populations and how can it be spatially represented in the Iberian Peninsula, the geographical region where the species emerged. Answering these questions will help to understand previous literature where different rabbit subspecies were described and evaluate their validity. The third question will focus on the reconstruction of the remarkable geographic expansion of European rabbits since historical times. Still related with this question is the analysis of the domestication process and how important it was in the determination of present-day patterns of genetic diversity in domestic breeds. Finally, the last question will ask if the introduction of devastating viral diseases like myxomatosis in the 1950s and the rabbit haemorrhagic disease in the 1980s left an imprint in the genetic structure of European rabbit populations.

How is Genetic Diversity Partitioned between Rabbit Populations?

The first genetic analyses of European rabbit populations were of very limited use and did not incorporate Iberian samples (e.g. Richardson et al. 1980; Peterka and Hartl 1992). It was only in the beginning of the 1990s that a group of French and Tunisian researchers described two very divergent mtDNA lineages within rabbit populations and further indicated that one of the lineages was only detected in southern Spain (Biju-Duval et al. 1991). Subsequently, the same group extended the analysis to other populations (Monnerot et al. 1994), but comprehensive studies of mtDNA variation appeared only recently (Branco et al. 2000, 2002). It is today very clear that diversity of rabbit populations at the mtDNA molecule is clustered in two clades that may have diverged as early as the transition Pliocene/Pleistocene (that is, between 2 and 2.5 Ma). It is also clear from the data that diversification within each mtDNA lineage started to accumulate more or less simultaneously, some 200,000 to 300,000 years ago (Branco et al. 2000). The very high mtDNA divergence together with an essentially parapatric distribution (see next section) leaded Branco et al. (2000, 2002) to hypothesize that the two lineages resulted from the allopatric differentiation of two rabbit groups that recently came into contact. This interpretation is in general agreement with multiple phylogeographic studies available in Europe, suggesting that many different species were fragmented and isolated during the climatic oscillations of the Quaternary (Hewitt 2004). However, an alternative hypothesis is that the mtDNA genealogy does not represent the evolution of rabbit populations but of the molecule itself. Recent work based on simulation studies has strengthened this hypothesis and highlighted the many limitations associated with the sole use of mtDNA markers in inferring the evolutionary history of species (e.g. Irwin 2002).

In our laboratories we have been developing a large-scale genetic analysis of rabbit populations based on allozyme polymorphism. In a recent review of previous work, Ferrand and Branco (2007) were able to show that protein diversity is also partitioned into two main population groups, in agreement with mtDNA data. In contrast with mtDNA, however, allozyme studies do consist in the simultaneous analysis of multiple independent markers scattered in the genome, thus giving strong support to the notion that present-day rabbit populations resulted from the isolated evolution of two groups during a considerable period of time. In such a scenario, the patterns obtained for protein polymorphisms and mtDNA can be considered as essentially concordant. One way of measuring the amount of genetic diversity that results from the putative historical split of rabbit populations in two groups is the G_{st} statistic (Nei, 1973). Ferrand and Branco (2007) estimated this value as 15.1%, which is moderately high but very far from the value of 78% estimated from the mtDNA data of Branco et al. (2000) using an equivalent statistic. It is likely that the progressive admixture between divergent population groups is much higher for autosomal markers than for mtDNA due to the 4:1 ratio in effective population size, thus resulting in the apparent discrepant values mentioned above. For example, Campos et al. (2007) analysed the distribution of genetic variation at the nuclear HBB locus and found an $F_{st} \approx 0$ between the two divergent rabbit populations, but suggested that in some point of their history that value could have been close to one. This fact could be the result of extensive admixture, eventually promoted by balancing selection, between two previously allopatric and alternatively fixed lineages at that locus.

Recent analysis of the two other genomic compartments in rabbit populations, the Y- and the X-chromosomes, confirmed the hypothesis that a significant fraction of the species diversity is due to the differences accumulated between two major groups of populations. In first place, the molecular analysis of the *SRY* region showed evidence for the occurrence of two divergent lineages separated by multiple point mutations (Geraldes et al. 2005). Subsequently, Geraldes and Ferrand (2006) verified that most of the genetic diversity observed at the *SRY* locus is due to differences between the two previously identified rabbit population groups. In second place, Geraldes et al. (2006) performed a detailed analysis of four independent markers located on the X-chromosome and described two divergent lineages in all of them. Additionally, by using coalescent simulations, they were able to show that those lineages had necessarily to result from the allopatric evolution of two divergent population groups of the European rabbit during a considerable period of time.

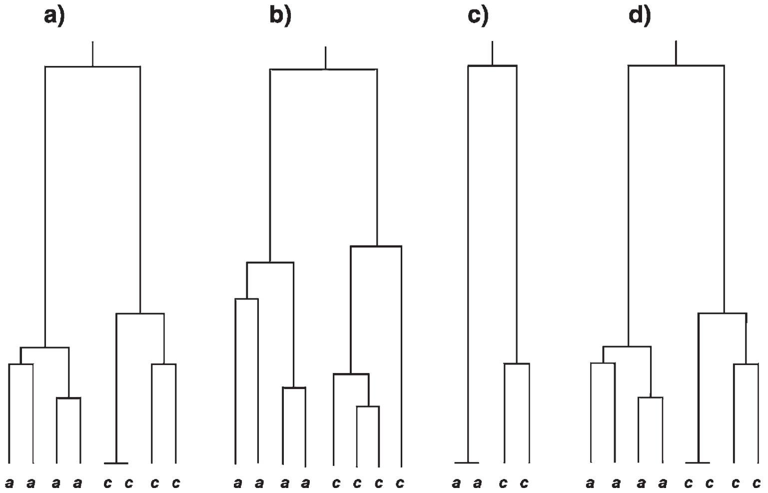

Fig. 2 Genealogical trees obtained from molecular markers at each of the four genomic compartments studied in rabbit populations: mtDNA (**a**), allozymes (**b**), Y-chromosome (**c**) and X-chromosome (**d**). In cases A, C and D, representative sequences were downloaded from GenBank, while for B allelic frequencies were obtained from Ferrand and Branco (2007). A remarkable and concordant deep split between two lineages is observed in all four genealogies. The lowercase letters *a* and *c* represent data from the *O. c. algirus* and *O. c. cuniculus* subspecies, respectively

Taken together, genetic evidence obtained from the four genomic compartments in rabbit populations (Fig. 2) very strongly suggests that two groups of this species have been evolving in allopatry during a significant part of the Pleistocene. This partition of genetic diversity is compatible with the idea that two subspecies of the European rabbit can be considered, and probably correspond to *Oryctolagus c. algirus* and *O. c. cuniculus*. In addition, this same evidence suggests that all other described subspecies (e.g. *O. c. brachyotis* or *O. c. habetensis*) do not find any correspondent evolutionary scenario and should be abandoned. Finally, this pattern of a deep lineage split common to all four genomic compartments also suggests that the European rabbit is a mosaic of two highly divergent genomes.

How is Genetic Diversity Spatially Distributed in the Iberian Peninsula?

As presented above, the Iberian Peninsula corresponds to the original geographical area where the species *O. cuniculus* emerged and stayed for most of its evolutionary history. It is thus especially relevant to perform a detailed analysis of the spatial patterns of genetic diversity for the four genetic compartments described in the previous section. The observation of both concordant and discordant patterns for those different types of markers can offer invaluable insights into the history of the rabbit species.

The first comprehensive dataset to be described in the literature was that of Branco et al. (2000), which regularly sampled rabbit populations from the whole Iberian Peninsula and using an RFLP approach showed that one mtDNA lineage was almost fixed the Iberian southwest, while a second lineage occurred mostly on the Iberian northeast. The few exceptions found were explained as possible introductions resulting from the frequent restocking operations for hunting purposes. In addition, the same authors described a contact zone that bisected the peninsula in a northwest-southeast direction and suggested that it resulted from the post-glacial expansion of two rabbit populations that persisted in isolation in two relatively small glacial refugia within the Iberian Peninsula. These hypotheses were later tested by the same authors (Branco et al. 2002) through the use of nested clade phylogeographical analysis (Templeton et al. 1995) and mismatch analysis (Slatkin and Hudson, 1991) and confirmed previous interpretations. Clearly, most of the mtDNA diversity was located in putative glacial refugia, and populations in the centre and north of the peninsula exhibited depleted levels of diversity due to recent geographical expansions. When the distribution of mtDNA lineages is displayed in a geographic map, a clear southwest-northeast gradient is observed (Fig. 3a).

A very similar gradient is observed when the protein data collected by Branco (2000) is analysed under the STRUCTURE software (Pritchard et al. 2000) and then displayed on a map (Fig. 3b). To do this, we searched for $K = 2$ in the STRUCTURE program (meaning that genetic diversity obtained for a set of 20 polymorphic proteins is interpreted according to the occurrence of two clusters of populations; see Pritchard et al. 2000) and identified each individual as belonging to population one or population two. The concordance of results observed for the mtDNA molecule and the set of polymorphic proteins clearly suggests that similar historical mechanisms shaped the spatial patterns detected for those two different types of molecular markers.

Confirmation for this type of spatial pattern is obtained from the analysis of the Y- and X-chromosome data, which are shown in Fig. 3c and d, respectively. Geraldes and Ferrand (2006) studied the distribution of the two divergent Y-chromosome lineages using an RFLP protocol and verified that the observed pattern very closely resembles that described for the mtDNA. Accordingly, most of the genetic diversity found in that genomic compartment is again the likely result of the evolution of two population groups in isolation. Hence, the evolutionary trajectories of both male and female lineages are essentially congruent and give support to the interpretation of a common historical process shaping diversity patterns in uni-parental molecular markers. In addition, Geraldes et al. (2006) studied four unlinked markers along the X-chromosome and found

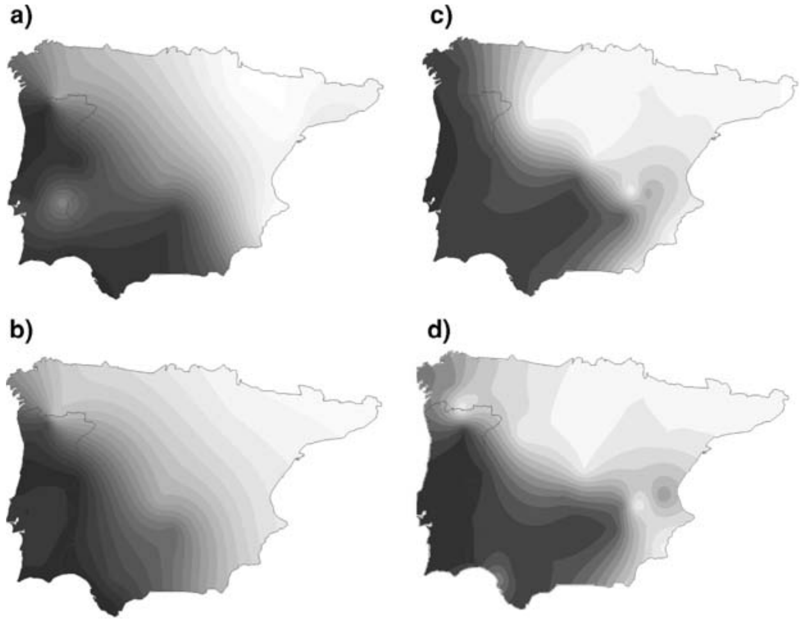

Fig. 3 Spatial analysis of patterns of genetic diversity at each of the four genomic compartments studied in Iberian rabbit populations: mtDNA (**a**), allozymes (**b**), Y-chromosome (**c**) and X-chromosome centromeric region (**d**). In cases A, C and D, haplotype frequencies were obtained from Branco et al. (2000), Geraldes and Ferrand (2006) and Geraldes et al. (2006), respectively, while for B allelic frequencies were obtained from Ferrand and Branco (2007). Interpolation and maps were prepared with the help of the SURFER software package. All gradients show essentially the same notorious southwest-northeast cline, but a smoother transition is observed for allozymes, while a more abrupt one is visible for both the Y- and the X-chromosomes

evidence that adds support to this scenario. This was particularly clear for the centromeric loci which, together with low levels of polymorphism and recombination, also showed low levels of introgression (see map in Fig. 3d).

Taken together, these data show that a major spatial component summarizing the patterns of genetic diversity exhibited by European rabbit populations in the Iberian Peninsula is the likely consequence of the long-term persistence of two isolated groups in glacial refugia during the Pleistocene. Moreover, it is also clear from the data that genomic regions with low or without recombination introgress much less that genomic regions that show regular or high recombination levels. This means that while the rabbit genome exhibits a widespread molecular signature of a mosaic nature, the recognition of a strongly marked spatial pattern is essentially restricted to particular genomic regions like the mtDNA molecule, the Y-chromosome or the centromeric regions of the X-chromosome.

What can we Infer about the Geographical Expansion of the Rabbit from Genetic Data?

The geographical expansion of the European rabbit is remarkable among mammals (Thompson and King 1994). Benefiting greatly from the direct transport by humans and the induced changes in habitats, rabbits expanded from the Iberian Peninsula in historical times, and occupied North Africa, most of Continental Europe, the UK, parts of South America, Australia, New Zealand, and hundreds of islands all over the world. Later, rabbits were domesticated and found immediate success as a source of protein for food, as well as for fur and wool.

Molecular markers are very useful tools to reconstruct the history of species geographical expansions using inference methods after the genetic characterization of extant populations. This is particularly true for the rabbit, where the availability of different types of genetic data greatly facilitates the task. On the other hand, the possibility of amplifying DNA from sub-fossil bones recovered from caves or other archaeozoological sites additional gives a temporal perspective that may offer valuable insights in the context of recent expansion events. Finally, combining these sources of information with historical documents provides the opportunity to have a more complete picture of rabbit geographical expansion.

A first debated question concerned with the species geographical expansion is the autochthonous or introduced character of the rabbit in North Africa. Conflictive interpretations exist in the literature (e.g. Dobson 1998; Surridge et al. 1999) and the fact is that the question was never properly addressed. Recently, Fonseca (2006) used a combination of mtDNA and microsatellite markers and concluded that North African rabbits result from a recent introduction of animals originating from southern Spain, and additionally show characteristics of admixture with domestic breeds. These results are in agreement with the idea that rabbits may have been introduced in North Africa by Phoenicians at the time of the first historical contacts established by navigating the Mediterranean, and also explain why the fossil record in that region is devoid of remains clearly belonging to *O. cuniculus*.

A second relevant question is related to the possible species persistence in southern France. It is clear from the fossil record that different *Oryctolagus* species once occurred in that region (see for example Lopez-Martinez 2008, this book), and that the same happened at various moments with *O. cuniculus* (Donard 1982). Thus, it is important to know if rabbits were able to persist in southern France across Pleistocene ice ages or, on the contrary, if they have recently arrived after the last glacial maximum. Hardy et al. (1995), using mtDNA polymorphism, and Queney et al. (2001), using highly variable microsatellites, both arrived at the conclusion that European rabbits colonized southern France post-glacially, exhibiting levels of diversity that are clearly lower than on the other side of the Pyrenees. The implication of these observations is that rabbit populations in that region were frequently submitted to cycles of extinction and recolonization, certainly because of climatic instability.

The subsequent colonization of the rest of continental Europe happened during the Middle Ages, and the analysis of protein polymorphism (Ferrand and Branco 2007), immunoglobulin allotypes (van der Loo et al. 1987, 2001) and mtDNA diversity in both extant and extinct populations (Hardy et al. 1995) indicate that this process was a mixture of a geographical expansion promoted by human-induced habitat changes together with the emergence of domestic breeds and the release of animals of mixed origin for hunting purposes (Callou 2003). Disentangling between the two processes is extremely difficult because rabbit domestication likely took place in southern France at approximately the same time (Callou 2003), but the future use of markers that are informative between ancestral wild populations and present-day breeds will certainly help to address this question.

The colonization of the UK is probably not connected with domestication as it happened earlier, resulting from an introduction by the Normans, during the 11th century (Callou 2003). This is clearly visible in some molecular markers like albumin (Ferrand and Rocha 1992) or carbonic anhydrase II (Branco and Ferrand 2003, unpublished results), as well as in some immunoglobulin allotypes (van der Loo 1987). UK rabbits were subsequently used as a source for the introductions that happened later in Australia and New Zealand, and the genetic analyses of those populations do confirm this hypothesis (Zenger et al. 2003; Ferrand and Branco 2007). Different are the populations that occur today in South America, and that strictly result from the release of domestic animals (Vieira 1993; Ferrand and Branco 2007).

One of the most remarkable aspects of rabbit colonization is its occurrence in more than 800 islands all over the world (Flux and Fullagar 1992; Flux 1994). Rabbits have been released on islands since historical times as a future source of meat for subsequent navigators and colonizers. It is thus very interesting to ask which sources were used by man for the purpose of establishing rabbits on islands. Mediterranean islands were probably among the first to be colonized by rabbits during the early stages of human routine navigation in that part of the world. These include the Balearics and the Tunisian island of Zembra, which may have been colonized as early as 1300–1400 B.C. (Reumer and Sanders 1984). In Zembra, the analysis of ancient-DNA (Hardy et al. 1994) together with the characterization of extant populations through the use of both mtDNA and allozyme markers (Ben Amor 1998) clearly suggested that rabbits were transported from the eastern coast of Spain, thus belonging to the subspecies *O. c. cuniculus*. Other rabbit populations within the Mediterranean islands remain to be studied but may have a similar origin.

On the contrary, the introduction of rabbits on the Atlantic archipelagos of Madeira, Azores and Canaries happened much later (early 15th century) and was thought to result from the initial steps of both Portuguese and Spanish discoveries. Due to the temporal coincidence of this human movement and the appearance of documents attesting the emergence of domestic rabbits, many papers suggest that those island populations are of feral origin. However, a preliminary study by Esteves (2003) on the genetic composition of the rabbit population from the island of Porto Santo, and then the comprehensive investigation of Fonseca (2006) in Madeira and most of the Azorean and Canary Islands, clearly showed that introduced rabbits were of wild origin, thus unequivocally exhibiting characteristics of the *O. c. algirus* subspecies. In addition, both studies indicated that island colonization was accompanied by a strong bottleneck and Fonseca (2006) further suggested that the rabbits of the three archipelagos exhibit the molecular signature of a common origin.

Taken together, these examples show the contrast between rabbit introduction histories in the Mediterranean and the Atlantic and highlight how interesting the analysis of many more cases of common movements between rabbits and man could be if adequately analysed with molecular tools.

How Strong was the Bottleneck that Lead to Rabbit Domestication?

It is well known that the process of domestication can impose a bottleneck effect on the genetic characteristics of both plants and animals due to the strong pressures of artificial selection determined by humans. However, it is also true that we still know very little about this subject because of a variety of reasons that include, for example, the extinction of ancestral wild populations (e.g. aurochs and wild horses), the difficulties of sampling wild populations (e.g. wild goats in Asia) or the lack of a proper study design. The rabbit is not one of these cases as abundant and easily available wild populations still occur today, and historical documents suggest that this species was domesticated in the Middle Ages, in southern France (Callou 2003). Notwithstanding this, a comprehensive study focusing on the genetics and demography of rabbit domestication is still lacking.

The detailed allozyme study of Ferrand and Branco (2007) together with the report of Queney et al. (2002) based on mtDNA variation and microsatellites are probably the best available examples of investigations addressing the question of how evident is a bottleneck associated to the history of rabbit domestication. Both studies reveal that genetic diversity of domestic breeds is a very small subset of that exhibited by European rabbit populations in the Iberian Peninsula. However, aspects as the genetic structure of rabbit populations, its geographical expansion and the genomic compartments analysed, among others, should be properly taken into account before considering an answer to the question. For example, mtDNA and the Y-chromosome are highly structured geographically, while allozymes are extensively admixed between divergent rabbit population groups in Iberia. On the other hand, French populations resulted from a recent and post-glacial expansion of rabbits from northeastern Spain that originated a considerable loss of genetic diversity

(Queney et al. 2001). When all these aspects are taken into consideration, the major result that emerges is that all domestic breeds are very similar genetically, and also do not show important differences when compared to their probable ancestors, French European rabbits. One explanation for this fact is that man sampled multiple times in wild populations (Queney et al. 2002) and kept genetic variability very high, as also described for other domesticated species (Vilà et al. 2005). In addition, the fact that commercial and selected strains apparently exhibit similar levels of genetic diversity could be the result of permanent exchanges between breeders (Queney et al. 2002).

While precise estimates of how much genetic diversity was lost during the domestication bottleneck, or how long did the bottleneck last, need further studies, available data suggest that most of the genetic variability present in French European rabbits also characterize domestic breeds. The recent availability of a draft of the rabbit genome and the future analysis of genes possibly implicated in the domestication process will certainly improve our understanding of rabbit evolution.

Can we Find a Signature of the Impact of Recently Introduced Diseases in the Genetic Diversity of Rabbit Populations?

The introduction of both myxomatosis and the rabbit haemorrhagic disease (RHD) had a dramatic impact in rabbit populations. Myxomatosis, for example, is described as having caused mortalities over 99.9% in some populations immediately after the release of the virus, in the mid 1950s of last century (Fenner and Fantini 1999). It is thus possible that this strong demographic bottleneck could have left an imprint in the genetic structure of rabbit populations. In agreement with this prediction, Surridge et al. (1999) analysed European rabbits from the British East Anglian region and argued that the genetic structure of present-day populations is the combined result of social structure and genetic drift acting on bottlenecked populations after myxomatosis, thus erasing any previous historical structure.

Population genetics theory predicts that rare alleles or haplotypes are the first to disappear from populations submitted to bottlenecks (Nei 1987). This property can thus be used to test this prediction in European rabbit populations. In fact, populations in the original distribution area of the species show a very high frequency of rare mtDNA haplotypes (Branco et al. 2002) or alleles at allozyme (Ferrand and Branco 2007) and microsatellite loci (Queney et al. 2001). In addition, multiple estimates of genetic diversity parameters suggest that rabbit populations in Iberia show very high levels of polymorphism when compared to most mammalian species (Ferrand and Branco 2007; Geraldes et al. 2006). This is in clear contradiction with the arguments of Surridge et al. (1999), and indicates that independently of the type of molecular markers employed to study rabbit populations, the impact of the viral diseases is hardly visible in the present-day genetic structure of the species. A more likely interpretation for the data described by Surridge et al. (1999) in East Anglian rabbits is that a rapid population expansion happened after introduction, retaining most of the genetic diversity and leading to the absence of population differentiation within the UK. A similar argument was invoked by Zenger et al. (2003) when explaining why the well-documented pattern of rabbit population expansion in Australia through a series of sequential founder events is not reflected in the patterns of genetic diversity.

Queney et al. (2000) took a different perspective and addressed this question experimentally benefiting from the fact that a French rabbit population has been the object of long-term ecological studies. Specifically, those authors estimated genetic diversity using both allozymes and microsatellites before and after the emergence of the RHD and verified that the well-documented demographic bottleneck (mortality rate was estimated at approximately 90%) induced no changes in the genetic composition of the population. Queney et al. (2000) thus concluded that in spite of high levels of mortality, it is likely that past epizootics had little or no impact in the genetic diversity of rabbit populations.

Different from the general demographic consequences resulting from the introduction of highly virulent diseases is the increasing genetic resistance of rabbits to both myxomatosis and RHD. In concordance with other diseases investigated in other species, it is possible that some relevant alleles in a few genes are contributing to this growing genetic resistance. This possibility is strengthened by the fact that the initial selection coefficients should have been extremely high. While candidate genes are available for both myxomatosis (Lalani et al. 1999) and the RHD (J. LePendu, personal communication), the fact is that very little is presently known about this possibility. Recently, Carmo et al. (2006) analysed genetic variation in chemokine receptors 2 and 5 (CCR2 and CCR5) and found that *O. cuniculus* was unique among Lagomorphs in the sense of exhibiting a gene conversion event of part of CCR2 into CCR5. These authors speculated that this fact could be associated to the extraordinary sensitivity of the rabbit to the myxoma virus, but more investigation is needed in this respect. In any case, it is clear that the rabbit offers unique characteristics to study the effects of natural selection caused by highly lethal diseases on genes that maybe involved in the emergence of genetic resistance.

Conclusions and Perspectives

This review on the genetics and evolutionary history of the European rabbit shows that the species exhibits very high levels of genetic diversity. While most of this diversity is allocated within populations, a significant part is due to differences between two highly divergent groups that have evolved in allopatry in the Iberian Peninsula, probably for most of the Pleistocene, and that correspond to the described subspecies *O. c. algirus* and *O. c. cuniculus*. These subspecies show a broad hybrid zone in central Iberia and expanded from refugia located in the southwest and the southeast of the peninsula, respectively. Interestingly, genomic regions that lack or have low levels of recombination also show low levels of introgression, while for most of the genome the rabbit exhibits a marked molecular dichotomy that resulted from an extensive admixture process between two differentiated groups. This pattern indicates that the two rabbit subspecies may correspond to an advanced stage of differentiation leading to its persistence even in the presence of high levels of gene flow, additionally suggesting that some degree of reproductive isolation may already exist. Future studies on the admixture dynamics of this hybrid zone may shed light on which parts of the genome are under strong spatial selection and may act to prevent a complete merge of the two subspecies. On a more practical side, this scenario argues against the use of rabbit translocations as an adequate management tool, at least when both subspecies are involved.

Genetic data also show that the recent geographical expansion out of Iberia was accompanied by a significant depletion in genetic diversity, and that most of the subsequent colonization episodes were followed by rapid population growth and the concomitant retention of previous diversity. Consequently, European rabbits in the more recently occupied areas show similar genetic characteristics and much lower levels of genetic diversity than Iberian populations. On the other hand, domestication had a small impact in the overall diversity of breeds and essentially reflects the genetic composition of European rabbits present in southern France. In this respect, future studies involving the analysis of candidate domestication genes (e.g. coat colour genes) will certainly provide more insights into the history of rabbit domestication.

Finally, the recent availability of the rabbit genome will offer additional opportunities to investigate issues related with these and other aspects of the species evolution, including a growing genetic resistance to viral diseases or the more controversial possibility of associating hybrid genomes with their capacity to colonize new environments.

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Phylogenetic Aspects of Nuclear and Mitochondrial Gene-Pool Characteristics of South and North African Cape Hares (*Lepus capensis*) and European Hares (*Lepus europaeus*)

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Introduction

Hares and jackrabbits (genus *Lepus*) are a notoriously difficult group, taxonomically, due mainly to broad phenotypic variation within taxa and wide overlap of traditional morphological characters (e.g., Angermann 1965, 1983; Flux 1983; Flux and Angermann 1990) across groups. However, several recent studies have demonstrated that forms representing superficially similar phenotypes but distinct evolutionary units can be differentiated by thorough analyses of morphological and phenetic characters and with the use of appropriate statistics (e.g., Palacios 1989 for hares from the Iberian Peninsula and Riga et al. 2001 for *Lepus corsicanus,* Italian hare). On the other hand, conspicuous phenotype differences or significant morphological or morphometric distinction might not always indicate differentiation at higher evolutionary level. For instance, the many domestic rabbit (*Oryctolagus cuniculus f. dom.*) breeds with all their different sizes and phenotypes have been created only very recently in evolutionary terms by anthropogenic selection and are still capable to be interbred. Similarly, in the genus *Lepus* it is conceivable that more or less strong selective pressure on relatively few genes, such as coat color genes or genes controlling for body size, could have led to conspicuous phenotypic adaptation to local or regional environments in forms that might otherwise still interbreed when they meet (again) in the wild.

Molecular data suggest fairly old ancestry of the genus *Lepus* (e.g., Halanych and Robinson 1999; Robinson and Matthee 2005). However, the currently traced *Lepus* lineages might represent offshoots of ancient lineages that were typical of ancestral taxa such as *Trischizolagus*, *Serengentilagus*, or *Hypolagus* (albeit there is no way to test this hypothesis, as these genera are all extinct). In effect, fossil evidence suggests that the whole genus *Lepus* has experienced its major adaptive radiation only recently in evolutionary terms

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(approx. within the last 2–2.5 million years), and probably many forms (species) are of much younger age. Therefore, overall genetic differentiation among many taxa might be relatively small. Moreover, in accordance with late Pleistocene climate changes and concomitant environmental perturbations, recent molecular data suggest complicated evolutionary scenarios for at least some species or forms, including phases of secondary contact and introgressive hybridization, as well as incomplete lineage sorting and presence of shared ancestral polymorphism (e.g., Thulin et al. 1997a; Alves et al. 2003; Kasapidis et al. 2005; Melo-Ferreira et al. 2005; Wu et al. 2005; Thulin et al. 2006; Ben Slimen et al. 2007). Such complex patterns of molecular evolution might lead to paraphyly for certain taxa (comp. e.g., Pérez-Suárez et al. 1994; Ben Slimen et al. 2007) and wrong systematic conclusions, particularly if only a single molecular marker system with limited power of resolution is applied and/or only few samples are studied (Alves et al. 2006; Ben Slimen et al. 2007).

As for other parts of the world, evolutionary relationships and systematics of various forms of hares from Africa must be considered provisional (e.g., Angermann 1983; Flux 1983; Flux and Angermann 1990; Robinson and Matthee 2005; Hoffmann and Smith 2005). To address only some of the systematic uncertainties, Ellerman and Morrison-Scott (1951) acknowledged the separate species status of *Lepus atlanticus* in northwest Africa, whereas Petter (1959), based on morphological arguments (particularly body size), hypothesized that cape hares (*Lepus capensis* L., 1758) include various forms from East Africa and all forms from North Africa except for an isolated occurrence of African savanna hares (*Lepus crawshayi*, sensu Petter, syn. to *Lepus victoriae* Thomas, 1823, cf. Flux and Angermann 1990, but see Hoffmann and Smith 2005) in North West Algeria. Petter (1961) even included all forms of the European hare (*Lepus europaeus* Pallas, 1778) into *L. capensis*, and most later authors implicitly acknowledged the presence of *L. capensis* in North Africa (e.g., Setzer 1958 for Egyptian hares, Petter and Saint Girons 1972 and Pérez-Suaréz et al. 1994 for Moroccan hares). Based on phenotypic and morphological comparisons, Angermann (1965) suggested the presence of *L. europaeus* in addition to *L. capensis* in North Africa, but was later on (Angermann 1983) somewhat unsecure about northwest African forms, that she related tentatively to "*granatensis*". *Lepus granatensis* (Rosenhauer, 1856) from the Iberian Peninsula and the Balearic Islands was earlier included either in *L. capensis* or *L. europaeus*, but morphological and molecular data undoubtedly demonstrate that *L. granatensis* must be considered a species distinct from *L. europaeus* and *L. capensis* (e.g., Bonhomme et al. 1986; Palacios 1989; Pérez-Suaréz et al. 1994; Alves and Ferrand 1999; Alves et al. 2000, 2003). Ellerman and Morrison-Scott (1951) acknowledged the presence of *Lepus arabicus* Ehrenberg, 1833 in parts of North Africa (Libya), a form that is currently listed as a subspecies of *L. capensis* (see e.g., Wilson and Reeder 1993) or possibly represents a separate species (Hoffmann and Smith 2005). Petter (1961) also retained *L. arabicus* as separate species, albeit with its distribution restricted to the Arabian Peninsula. The above-mentioned African Savanna hare (*L. victoriae*) has recently been renamed as *L. microtis* Heuglin, 1865 by Hoffman and Smith (2005). However, herein we follow the conclusive arguments of Petter (1959) and Angermann (1965), and consider this latter name as "*nomen dubium*" (a note on this taxonomic issue will be published elsewhere).

In this work, we present molecular clues to the evolutionary relationships between African cape hares and European hares and test Petter's (1959, 1961) hypotheses that North African hares with simple grooves in the first upper incisors (i.e., all forms except those from the environs of Beni Abbes, Algeria, that are presently considered *L. victoriae*) belong to *L. capensis*, and that this species also includes the European hare (*L. europaeus*). Specifically, we examine published molecular data in respect to nuclear and mitochondrial gene-pool differentiation among cape hares from South and North Africa and European hares.

Overall close gene-pool relationships would correspond to the null hypothesis of conspecificity of all samples, whereas distinct gene-pool divergence would agree with the current systematic view of separate species (i.e., *L. capensis* for South Africa and *L. europaeus* for Europe). In addition, considerable molecular divergence between *L. capensis* from South and North Africa would suggest differentiation on the species level, corresponding to the view of several earlier authors that considered some North African forms as separate species (see also Hoffmann and Smith 2005). To calibrate gene-pool divergence levels among our samples, we included allozyme data of mountain hares (*Lepus timidus* L., 1758) from three regions in Europe and sequences from diverse regions of Eurasia. On the one hand, mountain hares represent a "good *Lepus* species" (despite introgressive hybridization in wild populations, see below) with an evolutionary history clearly different from the samples of the "*L. capensis/europaeus* complex" (sensu Angermann 1983) and on the other hand they should also provide levels of within-species differentiation for comparison.

Comparative Analysis of Nuclear and Mitochondrial Gene-Pool Data

Recent molecular studies in the genus *Lepus* suggest a lower level of differentiation in nuclear gene-pools than in mtDNA. This seems to hold by and large for both within and between species comparisons (comp. e.g., Bonhomme et al. 1986; Hartl et al. 1993; Pérez-Suárez et al. 1994; Pierpaoli et al. 1999; Suchentrunk et al. 1999, 2000a; Alves and Ferrand 2000; Mamuris et al. 2001, 2002; Koh et al. 2002; Vapa et al. 2002, 2007; Alves et al. 2003; Suchentrunk et al. 2003; Fickel et al. 2005; Kasapidis et al. 2005; Sert et al. 2005; Waltari and Cook 2005; Wu et al. 2005; Ben Slimen et al. 2005; Estonba et al. 2006; Ben Slimen et al. 2007; Thulin et al. 2006). Therefore, we used both nuclear and mitochondrial gene-pool evidence. Specifically, we re-analyzed published multilocus allozyme and partial sequence data of the hypervariable domain 1 of the mtDNA control region of South African cape hares, hares from central Tunisia that are currently considered cape hares (e.g., Flux and Angermann 1990), and European hares.

Examination of Nuclear Gene-Pool Variability

To analyze nuclear gene-pool variability and differentiation among taxa we used published data of allelic variation at 29 structural (allozyme) gene loci (Hartl et al. 1993; Suchentrunk et al. 1999; Ben Slimen et al. 2005). All these data have been produced in our laboratory in Vienna under a standardized protocol (e.g., Grillitsch et al. 1992) by using marker samples in all gels for comparison of band patterns. Our set of loci was similar to that screened earlier in diverse hare species (e.g., Bonhomme et al. 1986; Grillitsch et al. 1992; Hartl et al. 1993; Suchentrunk et al. 1998, 1999, 2000a, 2001, 2003; Alves et al. 2001; Cervantes et al. 2002; Vapa et al. 2002; Ben Slimen et al. 2005; Sert et al. 2005); it encompassed the following 13 monomorphic loci (locus acronym, E.C. number, and locus in parentheses): lactate dehydrogenase (LDH, 1.1.1.27, Ldh -1), malate dehydrogenase (MOR, 1.1.1.37, Mor -1), malic enzyme (MOD, 1.1.1.40, Mod-1), catalase (CAT, 1.11.1.6, Cat), superoxide dismutase (SOD, 1.15.1.1, Sod-1,-2), purine nucleoside phosphorylase (NP, 2.4.2.1, Np), aspartate aminotransferase (AAT, 2.6.1.1, Aat-1, -2), hexokinase (HK, 2.7.1.1, Hk-1, -3), fumarate hydratase (FH, 4.2.1.2, Fh), and aconitase (ACO, 4.2.1.3, Aco-2). The 16 polymorphic loci are listed in Table 1. We used diverse software packages (Genetix, vers. 4.02, Belkhir 1999; Arlequin 3.01, Excoffier et al. 2005; Phylip pc package, Felsenstein 1995; PCO program, Anderson 2003) to calculate allele frequencies, linkage disequilibrium between polymorphic loci, diverse genetic distances for pairwise comparisons of differentiation among populations/taxa, to construct dendrograms of genetic relationships, to perform a metric principal coordinate (PCO) analysis of the Cavalli-Sforza-Edwards (CSE) chord distance matrix, and to assess the relative amount of nuclear gene-pool variability attributable to populations or groups of populations. In addition, we run assignment tests for each individual based on a Bayesian approach (Rannala and Mountain 1997, Paetkau et al. 2004 simulation algorithm, as implemented in GeneClass 2.0.g, Piery et al. 2004) to check for proportions of correct population assignment.

Examination of Mitochondrial Sequence Variability

For studying levels and patterns of mtDNA variability and differentiation we used published CR-1 sequences available on GenBank from African cape hares, European hares, and mountain hares. We selected a segment of 415 bp that allowed the alignment of 33 South African cape hare sequences, 60 European hare sequences, seven sequences of cape hares from north-central Tunisia, and 18 Eurasian mountain hare sequences (for accession numbers, provenances, and references see the appendix). The European hare sequences represented two phylogenetic groups of lineages, one occurring in several eastern Mediterranean islands and in some hares from Bulgaria (A-clade), and another one occurring in hares from most parts of Europe (B-clade) (see Kasapidis et al. 2005). The sequences were aligned with Clustal X (1.83) (Thompson et al. 1997), and a neighbor joining (NJ) dendrogram based on Tamura and Nei (1993) distances (TN93) was constructed with MEGA 2.1 (Kumar et al. 2001). The same program was used to evaluate the robustness of the NJ tree topology by bootstrapping (1,000 repetitions) and to confirm the NJ topology by running a maximum parsimony analysis (MP) without indels, with the close-neighbor-interchange option with search level 1 and with initial tree by random addition (10 repetitions) and 1,000 bootstrap repetitions. As a further alternative for portraying phylogenetic relationships among sequences, we subjected the TN93 distance matrix to a metrical PCO analysis (Anderson 2003). The resultant individual sequence coordinates were tested for variation among taxa by generalized least square regression (GLS), with a restricted maximum likelihood approach for variance homogeneity, separately for each dimension by using the S-Plus 6.2 program. For all series of tests, sequential Bonferroni corrections were applied to account for multiple tests (Rice 1989).

Patterns of Nuclear Gene-Pool Differentiation

Sixteen allozyme loci exhibited polymorphism (see Table 1 for allele frequencies and locus details). There was no linkage disequilibrium between any pair of polymorphic loci when tested separately in each taxon/population and when accounting for multiple tests. None of the samples revealed diagnostic alleles at any locus, and most common alleles were the common ones in all taxa or populations. However, mountain hares showed almost diagnostic alleles at the Sdh and the Acp-1 loci; occurrence of few alternate alleles in two European and Mountain hare populations was most likely due to introgressive hybridization (Suchentrunk et al. 2005). This allele pattern produced quite distinct gene-pool divergence between mountain hares and all samples of the "*L. capensis/europaeus* complex". However, nuclear gene-pool differentiation between Tunisian and European hares was only marginally higher than that found among populations of central European hares. South African cape hares showed a differentiation level that was clearly lower than that found between European and mountain hares. A NJ dendrogram based on CSE chord distances is given in Fig. 1.

The relatively close genetic relationships of South African *L. capensis*, presumed *L. capensis* from north-central Tunisia, and the central European *L. europaeus* populations were confirmed by the 2D PCO model, which explained all variation of the CSE chord distance matrix (see Fig. 2 for the

Table 1 Allele frequencies at polymorphic allozyme loci (*details in footnote*). See Hartl et al. (1993) for allozyme data of *L. europaeus*, Suchentrunk et al. (1999) for allozyme data of *L. timidus*, and Ben Slimen et al. (2005) for allozyme data of *L. capensis*. N = sample size

Locus	Allele	*L. capensis* (South Africa) $N = 9$	*L. capensis* (north-central Tunisia) $N = 45$	*L. europaeus* (range over five populations from central Europe) $N = 200$	*L. timidus* (range over three regions in Europe) $N = 200$
Sdh	100	1.0	1.0	1.0	0.0–0.041
	300	0.0	0.0	0.0	0.959–1.0
Ldh-2	100	0.944	0.935	1.0	1.0
	83	0.0	0.048	0.0	0.0
	105	0.056	0.016	0.0	0.0
Mor-2	100	1.0	1.0	0.964	1.0
	79	0.0	0.0	0.036	0.0
Idh-1	100	0.944	1.0	1.0	1.0
	121	0.056	0.0	0.0	0.0
Idh-2	100	0.611	0.978	0.933–1.0	0.321–0.954
	130	0.389	0.022	0.0–0.067	0.0–0.046
	83	0.0	0.0	0.0	0.0–0.679
Pgd	100	0.833	0.978	0.786–1.0	0.964–1.0
	170	0.0	0.0	0.0–0.052	0.0–0.36
	129	0.0	0.0	0.0–0.018	0.0
	117	0.0	0.0	0.0–0.036	0.0
	64	0.0	0.011	0.0–0.143	0.0
	79	0.111	0.0	0.0	0.0
	92	0.056	0.0	0.0	0.0
	69	0.0	0.011	0.0	0.0
Hk-2	100	1.0	1.0	0.969–1.0	0.973–1.0
	67	0.0	0.0	0.0–0.031	0.0–0.027
Es-1	−108	0.0	0.0	0.0–0.021	0.1–0.143
	−100	1.0	0.907	0.569–0.833	0.843–0.9
	−75	0.0	0.093	0.167–0.414	0.0
	−42	0.0	0.0	0.0–0.017	0.0
	−132	0.0	0.0	0.0	0.0–0.14
Pep-1	100	1.0	0.978	1.0	1.0
	83	0.0	0.022	0.0	0.0
Pep-2	100	0.0	0.856	0.768–0.875	0.0–0.053
	104	0.444	0.1	0.125–0.232	0.906–1.0
	114	0.556	0.022	0.0	0.0–0.058
	94	0.0	0.022	0.0	0.0
Acp-1	100	1.0	0.932	1.0	0.0–0.036
	115	0.0	0.0	0.0	0.964–1.0
	81	0.0	0.068	0.0	0.0
Mpi	100	0.444	0.956	0.914–1.0	0.929–0.964
	126	0.556	0.044	0.0–0.086	0.014–0.071
	77	0.0	0.0	0.0	0.0 –0.023

(*Continued*)

Table 1—Continued

Locus	Allele	*L. capensis* (South Africa) *N* = 9	*L. capensis* (north-central Tunisia) *N* = 45	*L. europaeus* (range over five populations from central Europe) *N* = 200	*L. timidus* (range over three regions in Europe) *N* = 200
Aco-1	100	1.0	1.0	1.0	0.979–1.0
	60	0.0	0.0	0.0	0.0–0.021
Mod-2	100	1.0	1.0	1.0	0.929–1.0
	110	0.0	0.0	0.0	0.0–0.071
Glud	100	1.0	0.944	1.0	1.0
	96	0.0	0.056	0.0	0.0
Es-D	100	0.978	1.0	0.75–0.875	0.810–0.929
	141	0.0	0.0	0.125–0.328	0.071–0.148
	72	0.0	0.0	0.0	0.0–0.042
	60	0.022	0.0	0.0	0.0

Isozyme/-system, abbreviation, E.C. number, and respective structural gene loci: sorbitol dehydrogenase (SDH, 1.1.1.14, Sdh), lactate dehydrogenase (LDH, 1.1.1.27, Ldh-2), malate dehydrogenase (MOR, 1.1.1.37, Mor-2), malic enzyme (MOD, 1.1.1.40, Mod-2), isocitrate dehydrogenase (IDH, 1.1.1.42, Idh-1,-2), 6-phosphogluconate dehydrogenase (PGD, 1.1.1.44, Pgd), glutamate dehydrogenase (GLUD, 1.4.1.3, Glud), hexokinase (HK, 2.7.1.1, Hk-2), esterases (ES, 3.1.1.1, Es-1; 4.2.1.1, Es-D), acid phosphatase (ACP, 3.1.3.2, Acp-1), peptidases (PEP, 3.4.11, Pep-1,-2), aconitase (ACO, 4.2.1.3, Aco-1), mannose phosphate isomerase (MPI, 5.3.1.8, Mpi)

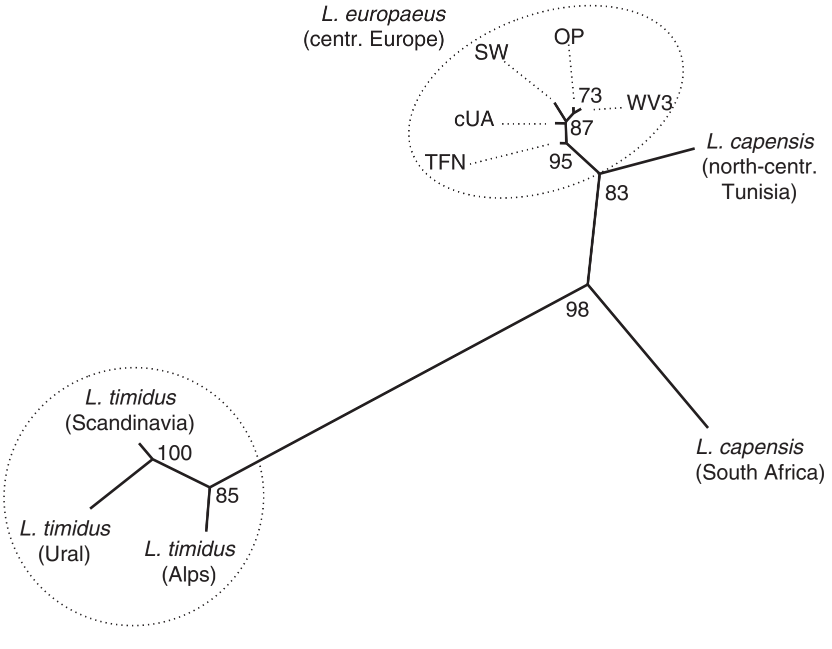

Fig. 1 Neighbor-joining dendrogram of *Lepus* taxa/populations studied presently, based on Cavalli-Sforza/Edwards distances calculated from 29 allozmye loci. Bootstrap support values (100 repetitions) above 50% are given at respective internal nodes. For European hare populations, see Hartl et al. (1993; cUA combines OWN, OWS, OIV, OKT); for mountain hare populations, see Suchentrunk et al. (1999); and for *L. capensis* populations see Ben Slimen et al. (2005)

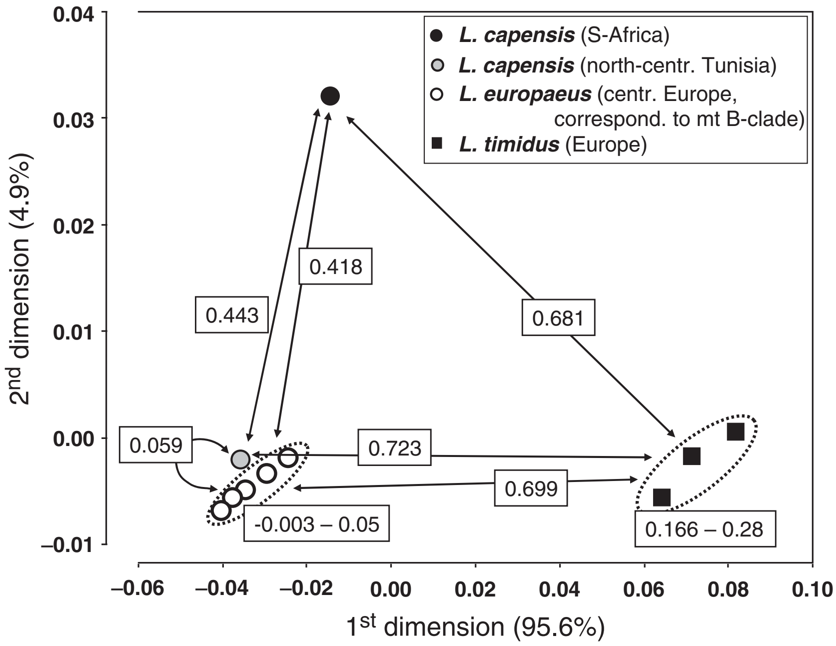

Fig. 2 Scatterplot of coordinates of hare taxa/populations as obtained from the 2D PCO analysis of the Cavalli-Sforza/Edwards chord distance matrix based on 29 allozyme loci. *Double-headed arrows* and associated values indicate mean pairwise Θ values (relative genetic differentiation) among taxa. Respective ranges of Θ values for among-population comparisons within central European hares and mountain hares are also given

scatterplot of population coordinate scores). Relative genetic differentiation (Θ values, see Fig. 2) was generally significant for pairwise comparisons between all taxa/populations, except for comparisons between populations of *L. europaeus* and between mountain hares from the Alps and Scandinavia, when based on Bonferroni corrections. An analysis of molecular variance (AMOVA) revealed that 12.57% of the total allozymic variance were due to variation between the *L. europaeus* and *L. capensis* (from both South and North Africa) samples (FCT = 0.126, n.s.), whereas 7.43% were due to differentiation among populations within those two groups (FSC = 0.085, $p < 0.00001$), and 80.0% were due to variation among individuals within populations (FST = 0.2, $p < 0.00001$). For calibration, a second AMOVA model, based on the comparison between populations of *L. europaeus* and *L. timidus*, revealed that 55.99% of the variation were due to separation into the two species (FCT = 0.559, $p = 0.0147$), 3.5% were due to differentiation among populations within each species (FSC = 0.0795, $p < 0.00001$), and 59.5% were due to variation among individuals within populations (FST = 0.595, $p < 0.00001$). The assignment tests were in essence concordant with the revealed pattern of gene-pool partitioning: all mountain hares were assigned correctly to one of the mountain hare populations, albeit only a small fraction was assigned correctly to the population; also, all South African cape hares were assigned correctly to the South African population, but only 68.9% of the Tunisian hares were assigned correctly, 8.9% were assigned to the South African cape hare population, and 22.0% were collected to one of the European hare populations. Reversely, only 49.0% of all European hares were assigned correctly to the species, with a very low proportion of assignment to the correct population, and the remaining 51.0% of European hares were assigned either to the Tunisian population (43.5%) or to the South African population (5.5%).

Differentiation of mtDNA CR-1 Sequences

A total of 117 sequences downloaded from GenBank (see Appendix) could be aligned for a 415-bp-long fragment of the mtDNA CR-1 and used for phylogenetic analysis. Due to the somewhat shorter alignment relative to several of those sequences published earlier, some of the downloaded original haplotypes were now identical. In the NJ dendrogram (Fig. 3), haplotypes of European hares from both the A- and B-clade clustered in two well-supported and closely related groups and the Tunisian haplotypes were also relatively close to the European hare haplotypes. In contrast, South African haplotypes were distinctly separate from both European hares and Tunisian hares. Surprisingly, Mountain hare haplotypes were closer to European hares and Tunisian hares than were the South African Cape hares. This tree topology was in essence confirmed by our MP analysis (175 variable sites, 147 parsimony informative sites, 28 singletons; iCI = 0.300156, iRI = 0.861709, for general explanation see e.g., Nei and Kumar 2000); for MP bootstrap values see also Fig. 3.

An eight-dimensional PCO analysis explained the total variation (101.1%) of the TN93 distance matrix, and the GLS analyses revealed significant differentiation of individual PCO scores of the sequences for the first five dimensions explaining 94.02%. The plots of the individual PCO values of the first four dimensions (Fig. 4) gave a pattern of distance relationships very similar to that in the NJ and MP trees.

Contrasting Patterns of Differentiation between Mitochondrial Lineages and Nuclear Gene-Pools

All analyses of partial CR-1 sequences demonstrate distinct mtDNA divergence among South African Cape hares (*L. capensis*), Tunisian Cape hares, and European hares (*L. europaeus*). However, this clear differentiation pattern is not fully paralleled by nuclear gene-pools. While our multilocus allozyme approach separates the South African Cape hares somewhat from the European hare populations, both in terms of absolute and relative genetic dif-

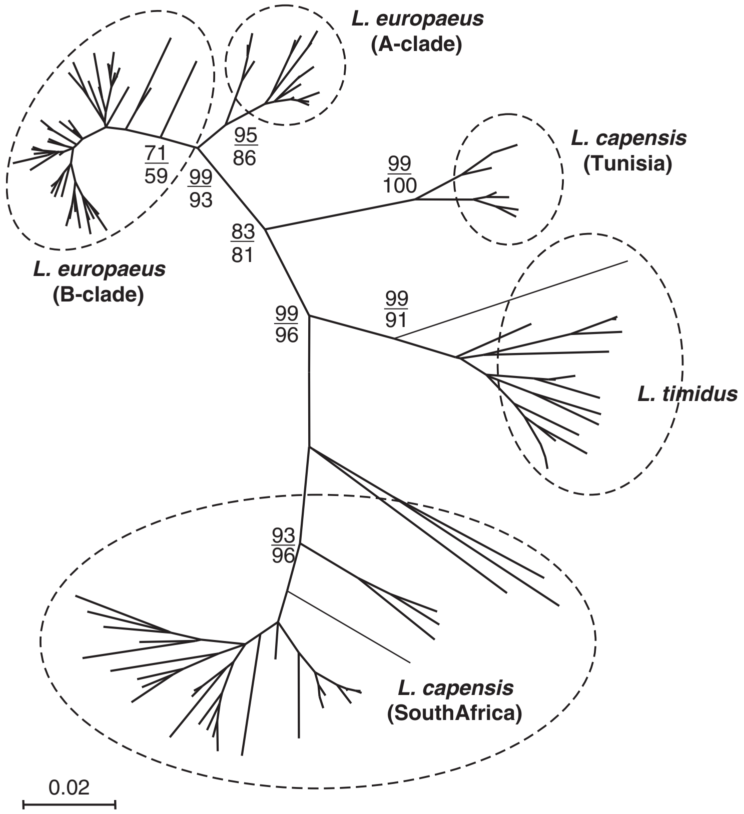

Fig. 3 NJ dendrogram of partial (410-bp) mtCR-1 sequences of South and North African *L. capensis*, European *L. europaeus,* and Eurasian L. *timidus* based on Tamura/ Nei (1993) distances. Bootstrap support values >50% are given for respective internal nodes for both the NJ (*upper values*) and the MP (*lower values*) majority rule consensus trees that had in essence the same topology as the presented NJ tree

ferentiation, hares from north-central Tunisia are fairly closely related to the European hares. North African hares are considered as belonging to *L. capensis* (e.g., Petter 1959; Flux and Angermann 1990; Wilson and Reeder 1993; but see Hoffmann and Smith 2005). The divergence level between South African Cape and European hares is approximately half of that between *L. europaeus* and *L. timidus* populations. The somewhat elevated divergence level between South African Cape hares and European hares is exclusively due to the pronounced differences in allele frequencies at some loci, but not due to alternately fixed alleles. In spite of the relatively high number of alleles at polymorphic loci that occur exclusively either in the South African Cape hares, the Tunisian hares, or the European hares ("private alleles"), the general nuclear gene-pool architecture is by and large the same for all these samples.

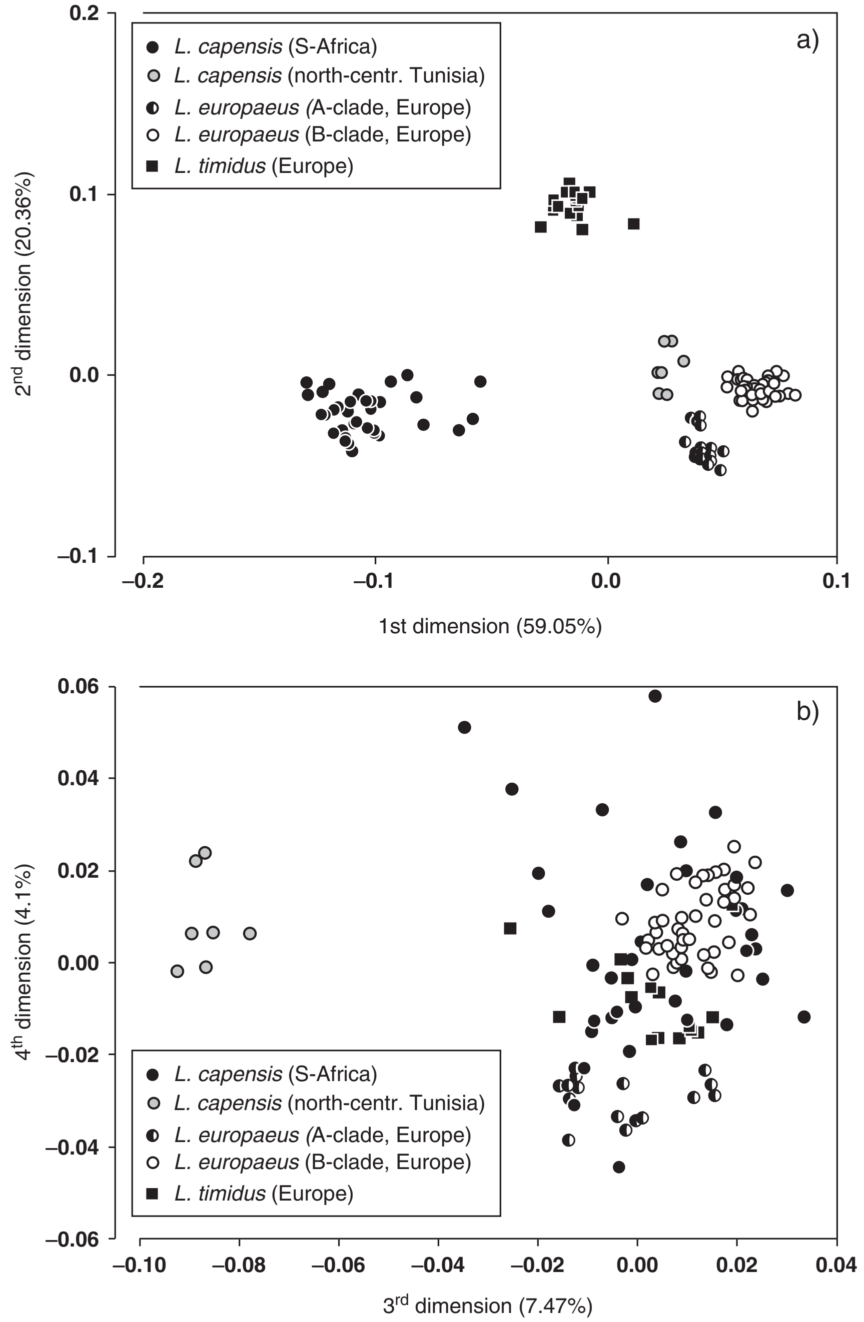

Fig. 4 Scatterplots of individual PCO scores of partial mtCR-1 sequence data as derived for the first two coordinate dimensions (**a**) and the third and fourth dimensions (**b**) of the eight-dimensional model

Alleles that are alternately fixed or almost alternately fixed occur only at two loci (Sdh, Acp-1) between *L. timidus* and *L. capensis* samples on the one hand and between *L. timidus and L. europaeus* on the other (Table 1). The few European hare-type alleles in Mountain hares at these two loci are considered as resulting from current or historic introgressive hybridization between European and Mountain hares from the Swiss Alps (Suchentrunk et al. 2005). Both the Sdh and the Acp-1 loci contribute to the high genetic differentiation between Mountain hares and all other samples.

Our set of allozyme loci identifies the Mountain hares as a clearly separate evolutionary unit, in accordance with their separate species status. Similar sets of loci revealed separate evolutionary lineages for "good hare species", such as Iberian hares (*L. granatensis*), Japanese hares (*Lepus brachyurus*), and Italian hares (*L. corsicanus*) (Alves and Ferrand 1999; Suchentrunk et al. 1999; Suchentrunk et al. unpubl. data). Thus, we consider our nuclear gene-pool results, based on 29 unlinked allozyme loci, appropriate for inferring evolutionary relationships among our samples; particularly because they rest on a wide range of rapidly, moderately, or slowly evolving structural gene loci (see also e.g., Richardson et al. 1986 for the general use of allozyme data for phylogenetic and speciation studies). On the contrary, mtDNA can be viewed rather as one linkage system, reflecting the evolution of the organelle rather than that of organisms. Therefore, mtDNA data might cause erroneous conclusions on species differentiation, if applied without concomitant data of multiple nuclear markers (e.g., Ballard et al. 2002; Ballard and Whitlock 2004). Inferences on phylogenetic relationships of *Lepus* species that have exclusively been drawn from mtDNA data (e.g., Pierpaoli et al. 1999; Waltari and Cook 2005; Wu et al. 2005), in spite of published evidence for various introgression scenarios for mtDNA in diverse hare species (see Thulin et al. 1997a; Alves et al. 2003; Melo-Ferreira et al. 2005), must therefore be considered preliminary at best.

Our finding that differentiation among South and North African Cape hares and European hares is clearly lower for the nuclear gene pool than for the mtDNA is in accordance with recent studies of European and Anatolian European hares. While nuclear gene-pool differentiation as assessed by allozyme and RAPD analysis indicated relatively little divergence between Anatolian and European populations on the one hand, and between Greek and Central European populations on the other, mtDNA RFLP and sequence data indicated a clearly higher level of differentiation (cf. Mamuris et al. 2001; 2002; Suchentrunk et al. 2003; Kasapidis et al. 2005; Sert al. 2005). The discordance between nuclear and mtDNA is probably due to higher gene flow in males and more pronounced philopatry in females. For Central European hares, such a sex-specific difference in gene flow was evident even on small geographic scale between neighboring populations (Fickel et al. 2005); and it was also concluded from molecular data for South African Cape hares and Scrub hares (*Lepus saxatilis*) by Kryger (2002).

Regarding mtDNA data, there are many more *Lepus* control region sequences available on the GenBank than the ones presently used in our phylogenetic analysis. However, we aimed to compare patterns of nuclear and mitochondrial gene-pool differentiation among three phenotypically similar taxa (*L. capensis* from South and North Africa, and *L. europaeus*), rather than to develop a model of phylogenetic relationships within the genus *Lepus*. The present results clearly show distinct divergence of all analyzed mtDNA sequences into three monophyletic clades (i.e., South African *L. capensis*; North African *L. capensis;* and *L. europaeus* with two subclades) without any hint of paraphyletic lineages as regards the presently studied taxa. This might indicate that the mtDNA data in this study are free of possible cases of introgression or nuclear representations of mtDNA ("numts"). However, in view of the relatively high sequence divergence in the South African Cape hare clade and one divergent haplotype in the Mountain hare clade, we do not fully exclude the occurrence of numts.

Reticulate Evolution in the Genus *Lepus* and Consequences for Phylogeny Reconstruction

As pointed out by Alves et al. (2006) and Ben Slimen et al. (2007), a phylogenetic analysis within the genus *Lepus* must include nuclear evidence because of the possibility of unrecognized presence of introgressed mtDNA. Phases of reticulate evolution cannot be excluded for some species, and this could lead to erroneous conclusions, if phylogenetic inferences are based exclusively on mtDNA. Thulin et al. (1997a), Alves et al. (2003), and Melo-Ferreira et al. (2005) have demonstrated introgressive hybridization of *L. timidus* type mtDNA into *L. europaeus* and *L. granatensis*, and occurrence of foreign mtDNA in other *Lepus* species is very plausible (see e.g., Pierpaoli et al. 1999 for *Lepus starcki* (Ethiopian Highland hare) and *Lepus habessinicus* (Abyssinian hare); Alves et al. 2003; Ben Slimen et al. 2007). Preliminary data (Suchentrunk et al. 2005) reveal substantial bidirectional introgressive hybridization in both nuclear and mtDNA of wild living European hares and Mountain hares from Switzerland, but limited morphological consequences in higher generation hybrids. Introgression might be even more likely in cases of secondary contact of less differentiated (conspecific) gene-pools of hares, as found between the European and inferred Anatolian/Middle Eastern mtDNA lineages of European hares (Kasapidis et al. 2005), and such evolutionary scenarios might inflate mtDNA variability within species. The presently compared South African *L. capensis* sequences show a relatively high phylogenetic heterogeneity. Their range of pairwise distances (up to 15.8%) is somewhat greater than that of the Tunisian hares and that found between the two clades of European hares from the eastern Mediterranean (A-clade: from inferred Anatolian Pleistocene range, B-clade: southeastern European Late Pleistocene range). The latter two clades from Southeast Europe and the Aegean Islands show an average nucleotide divergence of 6.6% (Kasapidis et al. 2005).

Most likely, the increased sequence heterogeneity of the South African *L. capensis* sequences results from a distinct phylogeographic partitioning in Africa (Kryger 2002). However, even for one local sample from the northern Cape Province Ben Slimen and Suchentrunk (in press) revealed a substantial divergence level (up to almost 8%) of mitochondrial lineages. Relatively high levels of differentiation (up to 7.3% sequence divergence) were also found for partial control region sequences of other hare species, such as Palaearctic Mountain hares (*L. timidus*) by Thulin et al. (1997b), and this was interpreted as resulting from the existence of ancestral lineages in a (little structured) continuous population that existed during the last glaciation in Europe. Similarly, persistence of shared ancestral polymorphism could not be excluded for Palaearctic lineages of Mountain hares (Ben Slimen et al. 2007). On the other hand, inflated mtDNA variability and hence a tendency towards taxonomic inflation might result from continuous large effective population sizes of tropical species in contrast to species from more northern latitudes that might have experienced bottlenecks during the climatic changes in the (Late) Pleistocene (Harris and Froufe 2005). Whether nuclear gene-pool differentiation of South African Cape hares shows a similarly high level of heterogeneity or fits the above hypothesized general mode of higher nuclear gene flow relative to gene flow in mtDNA of hares, or whether those South African hares currently considered cape hares should on the contrary be split into separate species, remains to be studied by nuclear gene-flow analyses and other biological characters. Our preliminary allozyme, microsatellite, and mitochondrial sequence data of some cape hares, however, suggest conspecificity despite quite substantial sequence divergence in one (conspecific) South African population (Ben Slimen and Suchentrunk, in press).

Revival of Petter's (1961) Hypothesis of Conspecificity of *L. europaeus* and *L. capensis*

The presently found close nuclear gene-pool relationships between South African and Tunisian cape hares and central European hares fit the hypothesis of conspecificity of *L. capensis* and *L. europaeus*, put forward by Petter (1961) on morphological grounds. Similarly, Angermann (1965), also based on morphological comparisons, considered the occurrence of European hares in parts of North Africa. Later on she was still not clear about some forms of the *L. capensis* / *L. europaeus* complex, and wrote: "*L. capensis* s. l. may consist of parapatric forms in various stage of divergence—subspecies, semispecies or allospecies" (Angermann 1983). However, in a provisional summary of the genus *Lepus*, Flux and Angermann (1990) considered European hares absent from Africa. Flux (1983) also pointed towards the taxonomic uncertainty of forms of *L. capensis* and *L. europaeus*, and European hares were indeed accepted as subspecies of *L. capensis* for a while by checklists or various authors during the second half of the 20th century (see e.g., checklist in Myers and McInnes 1981). Petter (1959, 1961) also included all hares from North Africa (except one isolated population of *L. victoriae* in the region of Beni Abbes, NW Algeria) into *L. capensis*, and our data of Tunisian hares (see also Ben Slimen et al. 2005) support his hypothesis. Moreover, mtDNA PCR-RFLP data (Ben Slimen et al. 2006) suggest close genetic relationships among South African Cape hares, north-central Moroccan and north-central Tunisian hares. In addition, partial CR-1 sequence data (Ben Slimen et al. 2007) and microsatellite data for 11 loci (Ben Slimen et al. unpubl. data) indicate close phylogenetic association between Tunisian and desert hares from northwestern Egypt and relatively high gene flow among those populations. Certainly, we cannot exclude the possibility that the little overall divergence indicated by the presently studied allozyme loci might to some degree be due to concordant selection effects at those loci, and that the differentiation pattern revealed by eleven microsatellite loci (Ben Slimen et al. unpubl. data) is due to possible length homoplasy for some of those loci. Also, even single genes that have currently not been studied might indicate a different species and could thus lead us to a false conclusion. But the most parsimonious interpretation of our results on nuclear gene-pool relationships would be that South African and Tunisian Cape hares and probably also European hares are conspecific, thus matching Petter's (1959, 1961) hypothesis.

Gene-Pool Relationships and Species Concepts

In contrast to the allozyme results, the present results on mtDNA differentiation are not congruent with Petter's (1961) hypothesis of conspecificity of *L. capensis* and *L. europaeus*. However, we suggest that the presently revealed distinct evolutionary divergence of the highly variable mtDNA control region fragment between South African Cape hares, North African hares, and European hares might result from regional anagenesis within a network of geographically separated populations that are still cohesive in evolutionary terms, fitting an "interbreeding species concept" (Lee 2003). Screening many contiguous populations of hares that are currently considered *L. capensis* for mtDNA variation might reveal more or less continuous mtDNA gene-pool among those hares that are currently included in *L. capensis*, *L. europaeus*, and perhaps also *L. tolai*, albeit there is apparently a (relatively recent?) distributional gap between South and East African populations (e.g., Flux and Angermann 1990). A high level of mtDNA variability but relatively low differentiation in nuclear DNA is rather the rule for hares from north-central Tunisia and for European hares from diverse parts of Europe and Anatolia (Mamuris et al. 2001, 2002; Suchentrunk et al. 2003; Kasapidis et al. 2005, Sert et al. 2005; Ben Slimen et al. 2005, 2006, 2007; see also Fickel et al. 2005). Alternatively, mitochondrial lineages of "*L. capensis* sensu latu" (forms included in the cape hare by Flux and Angermann 1990) might represent regional offshoots from an ancestral gene pool of a basal *Lepus* species that gave raise to the evolution of all modern *Lepus* species. Such an interpretation is backed up by a more comprehensive comparison of mitochondrial DNA haplotypes by Ben Slimen et al. (2007).

In conclusion, our comparison of nuclear and mitochondrial gene-pool differentiation suggests that *L. europaeus* might go (once again) into *L. capensis* L., 1758. Variation of external phenotypes (e.g., coat color types, external measurements) of these hares might be paralleled only at a low level of gene-pool differentiation. This was shown for Anatolian and Tunisian hares (Sert et al. 2005; Ben Slimen et al. 2005, 2007; see also Suchentrunk et al. 2000b), and preliminary mtDNA data of over 100 hares from different parts of Tunisia revealed only a little effect of coat color types (four types considered) on partitioning of sequence variance. Phenotypic characters such as body size, coat color, ear length, etc. might be under more or less strong selection by environmental characteristics. A good example is given by domestic rabbits that have evolved an enormous amount of phenotypic forms including coat color and size and shape characteristics within a very short period of evolutionary time (albeit under very strong selection by breeders). European hares that were introduced from Britain to New Zealand less than 150 generations ago have already adapted in their body size to the new environments following Bergman's rule (Flux 1990) and European hares from our breeding station in Vienna exhibit already significant reduction in skull (body) size after less than 20 years (unpubl. data). In mammals, many (quantitative, epigenetic) characters are likely controlled by a relatively small number of structural gene loci. If such phenotypic characters of cape and European hares are not linked to representative gene-pool compartments or to genes that are important in the context of reproduction, overemphasizing them in phylogenetic analyses might lead to a portrayal of habitat characteristics to which the hares were exposed in their local evolution, rather than a picture of overall evolutionary relationships.

To test Petter's (1961) hypothesis of conspecificity of Cape and European hares, gene-flow analyses are necessary for many nuclear markers (preferentially allozymes, nuclear gene sequences, microsatellites) of many neighboring populations in Africa, the Middle East and Europe, hence, a population genetic approach would be necessary. Inferences coming from mtDNA phylogeography certainly will help to understand historic population demography and relationships in these hares (see e.g., Kryger 2002; Kasapidis et al. 2005). Given that even *Lepus* species with very divergent mtDNA, such as Iberian, European and Mountain hares, may exhibit a considerable level of introgressive hybridization in natural populations (Thulin et al. 1997a; Alves et al. 2003; Melo-Ferreira et al. 2005; Suchentrunk et al. 2005; Thulin et al. 2006; Ben Slimen et al. in press), we might expect a gradual change of gene-pool characteristics in chains of intergrading populations between South African *L. capensis* and *L. europaeus*, similar to "ring species" scenarios (see e.g. Irwin et al. 2005). The current distributional gap between southern African and more northern populations (e.g., Flux and Angermann 1990) renders the populations allopatric and excludes natural gene flow between the southern African Cape hares and presumed northern Cape hare populations per se. However, this distributional gap might be very recent in evolutionary terms, and gene-pools of South African Cape hare populations and those from north of the distributional gap might thus be similar, particularly if large effective population sizes were present before the gap has formed, and if no serious bottlenecks have occurred afterwards. Such a scenario would in principal not contradict an "interbreeding species concept" or a "cohesive species concept" (see e.g., Lee 2003). European hares from continental Europe and the British Isles (Suchentrunk et al. 1998, 2001) as well as the disjunct populations of Mountain hares from Scandinavia, Scotland, Ireland, and the Alps (see Thulin et al. 1997a; Suchentrunk et al. 1999) serve as examples of allopatric though conspecific populations, respectively. Our present view is in contradiction to the position of Hoffmann and Smith (2005) who tend to split "*L. capensis* sensu latu" into several species, based on the argument there would be indications of restricted gene flow in *L. capensis*. However, at least all nuclear gene-pool data (the present ones and those of Kryger 2002 on microsatellites of South African *L. capensis*) indicate a rather low level of relative genetic differentiation among populations (i.e., low pairwise F_{st} and R_{st} values). Despite distinct mitochondrial gene-pool separation, Kryger (2002) reported gene flow among populations at a level that translated into clearly more than one individual theoretically migrating per generation between populations. This would be theoretically sufficient to counteract genetic differentiation due to random genetic drift in the populations. In our opinion, the "*L. capensis/europaeus* complex" (as coined by R. Angermann 1983) represents an exciting taxon for studies on speciation and evolution in mammals. Its systematic resolution needs a combined approach of phylogenetics, phylogeography, and population genetics, based on various nuclear and mitochondrial markers, and including other biological characteristics, such as phenotypic and morphometric data.

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Appendix

List of sequences downloaded from GenBank and used for our alignment (sequences with nucleotide ambiguities were not considered)

Species	Accession number in GenBank	References
L. capensis (South Africa)	AF491353 to AF491385	Kryger et al. (2002), direct submission
L. europaeus (Bulgaria, Greece and various Aegean Islands, Cyprus, northern Israel)	AY466782 to AY466853; except AY466827, AY466798, AY466799, AY466800, AY466813, AY466823, AY466824, AY466833, AY466836, AY466838, AY466850, AY466851	Kasapidis et al. (2005)
L. timidus (Sweden, Norway, Scotland, Russia, China)	AY422309 to AY422325; except AY422318	Waltari et al. (2004)
	AJ287976 and AJ287977	Wu et al. (2005), direct submission
L. capensis (north-central Tunisia)	DQ207740 to DQ207746	Ben Slimen et al. (2007)

Population Ecology and Dynamics

The World of Pikas

Andrew T. Smith

Introduction

The 30 currently recognized living species of pika (Ochotonidae: Genus *Ochotona*) comprise approximately one-third of all lagomorphs. The Leporidae, in contrast, contains 32 species of hare (Genus *Lepus*) and 29 species of rabbit (multiple genera; Hoffmann and Smith 2005). Both traditional (morphological) and molecular evidence indicates that the Ochotonidae separated from the Leporidae during the Oligocene, approximately 37 Mya. The Ochotonidae originated in Asia, but had spread to North America by the late Oligocene. Throughout their Holarctic range, and even extending into northern Africa, pikas differentiated and became quite diverse during the Miocene. Modern pikas (*Ochotona*) first appeared in Asia during the early Pliocene and spread to North America by the mid-Pliocene. The only two pika genera to appear in historical times, *Prolagus* and *Ochotona*, appeared in Europe by the late Pliocene. Following the extinction of *Prolagus*, *Ochotona* became the only living representative of the family that had flourished with as many as 25 fossil genera (Dawson 1967; Erbajeva 1988, 1994).

Today's pikas are found primarily in the high mountains of western North America (two species) and across Asia (28 species; Smith et al. 1990; Hoffmann and Smith 2005). The "hotspot" for *Ochotona* is China, home to 24 species. No consensus has been reached on the systematics of *Ochotona*; in fact, no two revisions of all or part of the genus are remotely similar (Smith et al. 1990; Yu et al. 2000). Molecular approaches are beginning to unravel the Gordian knot of pika systematics, but much remains to be clarified (Yu et al. 2000; Niu et al. 2001, 2004). We do know that most pikas are remarkably similar in morphology and that they generally live in some of the most remote landscapes on earth.

The purpose of this review is to bring attention to pika biology by demonstrating some of the important issues in ecology, behavior, and conservation that can be addressed by studying pikas. It is distressing that only three of the 174 presentations at the Second World Lagomorph Conference held in Portugal (1.7%) were focused on pikas, whereas 33% of papers should have been on pikas if they had been represented according to their species richness within

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the Lagomorpha (Alves and Ferrand 2004). In addition, most practitioners of the leporids fail to compare their results with the ochotonids. Similarly, while pikas are clearly “small mammals,” those studying the more abundant rodents also generally do not compare their findings with those of pikas. The comparative method is a powerful tool in biology, and the failure to utilize pikas as a robust and independent lineage for comparisons is regrettable. This review will show that pikas represent an exciting group of animals to study.

What is a Pika?

Pikas are small egg-shaped animals with prominent round ears and no visible tail. Pikas are fairly uniform in size with body mass ranging generally from 70 to 300 g among species. There is little sexual dimorphism. Pikas that have been studied have relatively high body temperatures, and most species appear to be quite sensitive to high ambient temperatures (Smith 1974a; MacArthur and Wang 1973, 1974). Most pikas are diurnally active. Like other lagomorphs, they do not hibernate. Pikas are generalized herbivores and they produce two types of feces like their leporid cousins—dry round hard pellets and a soft dark viscous caecotroph. Most species construct caches of vegetation during the short summer and live off of these stores (called haypiles) during the winter (Millar and Zwickel 1972; Conner 1983; Huntly et al. 1986; Dearing 1997). Another trait characteristic of pikas, but not of most other lagomorphs, is their propensity to vocalize (Conner 1985; Smith et al. 1986). Several morphological characteristics distinguish ochotonids from leporids: (1) small size (head-body length generally <285 mm, whereas leporids are all >300 mm); (2) the short round ears (<40 mm) compared with long ear pinnae (>60 mm) in leporids; (3) concealed tail versus short but evident tail in leporids; (4) skull without supraorbital process versus a skull with supraorbital processes in leporids; and (5) two pairs of upper premolars versus three pairs of upper premolars in leporids.

Pikas occupy two distinctly different habitats: rock piles and talus versus meadow and steppe, and our understanding of pika biology necessarily begins with a comparative examination of the life history characteristics expressed by rock-dwelling and meadow-dwelling pika species.

Rock-Dwelling Versus Meadow-Dwelling Pikas

All North American pikas and about half of the species in Asia live in rocky habitats and forage in adjoining meadows or patches of vegetation interspersed within the rocks; these are generally non-burrowing forms (Formozov 1981; Smith 1988). In contrast, approximately half of the Asian species occupy meadow or steppe habitats where they characteristically make burrows (Smith 1988). These habitat types are blurred in some cases; there are a few forms (such as the Afghan pika, *Ochotona rufescens*, and Pallas's pika, *Ochotona pallasi*) that occupy open habitats where they make burrows, but that can also be found in rocky areas. These "intermediate" species are not characterized by an intermediate suite of life history traits between those of rock and meadow-dwelling pikas; instead, they adopt the life history characteristics of meadow/burrowing pikas (Smith 1988). Some populations of rock-dwelling pikas live in what first seem to be atypical habitats such as piles of logs or moss covered boulders (common in the Northern pika, *Ochotona hyperborea*), old walls (Glover's pika, *Ochotona gloveri*; Chinese red pika, *Ochotona erythrotis*), or detritus left by human occupation (American pika, *Ochotona princeps*). In these cases, it is apparent that the pikas are keying in on the disruptive nature of the habitat, as their life history traits otherwise match those of pikas living in pure talus environments.

Elsewhere, I have reviewed in detail the species that fall into these two dramatically different eco-types of pika species (Smith 1981a, 1988; Smith et al. 1990). Pika species typically found in rocky habitats are the Alpine pika (*Ochotona alpina*), Collared pika (*Ochotona collaris*), Chinese red pika (*O. erythrotis*), Glover's pika (*O. gloveri*), Himalayan pika (*Ochotona himalayana*), Northern pika (*O. hyperborea*), Ili pika (*Ochotona iliensis*), Large-eared pika (*Ochotona macrotis*), American pika (*O. princeps*), Royle's pika (*Ochotona roylei*), and Turkestan red pika (*Ochotona rutila*). Typical meadow-steppe burrowing pikas include the Gansu pika (*Ochotona cansus*), Plateau pika (*Ochotona curzoniae*), Daurian pika (*Ochotona dauurica*), Kozlov's pika (*Ochotona koslowi*), Ladakh pika (*Ochotona ladacensis*), Muli pika (*Ochotona muliensis*), Nubra pika (*Ochotona nubrica*), Moupin pika (*Ochotona thibetana*), and Thomas' pika (*Ochotona thomasi*). In this review I will concentrate on comparisons between the rock-dwelling American pika and meadow-dwelling Plateau pika, two species on which I have worked extensively, because they present a clear contrast in ecology, reproduction, and behavior. Gliwicz et al. (2005) provide a comparison between the rock-dwelling pikas in North America and Asia.

Ecological Relationships

American pikas occupy individual territories at low density (generally 8–10/ha) on talus habitat (Smith and Ivins 1984; Smith and Weston 1990). There is no significant difference in territory size between males and females, although males are more likely than females to roam across the talus beyond the confines of their territory. Territory size remains remarkably constant across the geographic range of the species. Nearest-neighbor distances between centers of activity on territories range from about 14 to 30 m. Home ranges appear to be about twice the size of territories, although both measurements are sensitive to the method used for their determination (Smith and Ivins 1984; Smith and Weston 1990). There is a general trend for territories to be smaller when vegetation bordering the talus is more productive compared with larger territories in less productive settings (Millar and Zwickel 1972; Millar 1973). Additionally, territories of American pikas are more likely to be found near the talus/vegetation interface than deeper into the talus, where animals would have a longer distance to travel to feed or secure vegetation for their haypiles. One of the primary differences between the rock-dwelling pikas of North America and those from Asia is that Asian forms tend to live as pairs, although even in these situations there are few direct social interactions between the male and female of the pair (Kawamichi 1970; Smith 1981a; Nikolskii and Mukhamediev 1997; Gliwicz et al. 2005).

Plateau pikas, in contrast, live in social family groups on alpine meadows across the Qinghai-Xizang plateau (Smith et al. 1986; Smith and Wang 1991; Dobson et al. 1998, 2000). These family group territories consist of a traditional communal burrow system on which adult males and females live (in various combinations; see below) and their young of the year. Approximately 25 m separates the center of activity of a family group from that of each of the surrounding family groups. Regional density may approximate 300/ha following the reproductive season, although density at this time is highly variable among years. Density is also variable seasonally within single years, as high overwinter mortality knocks the population down to a low level just prior to the onset of reproduction (Wang and Smith 1988). This overwinter mortality is particularly severe in years with unusually deep snow, a situation that leads to strong inter-year differences in density (Wang and Smith 1988).

In summary, American pikas live at low density, but they are long-lived (some individuals live 6 years; Smith 1974b, 1978). These dynamics ensure that population density is relatively constant among years. Most Plateau pikas are short-lived (almost all breeding age animals are 1 year old) and populations fluctuate greatly on a seasonal and annual basis. Their populations may reach extremely high densities.

Reproduction

All aspects of reproductive performance in American pikas converge to yield a very low annual production of offspring (Millar 1973, 1974; Smith 1978). While two litters are initiated each breeding season, only one is successfully weaned. Normally the first litter is successful and the second litter is lost after parturition. Pika mothers put on fat during the gestation of their first litter and these energy resources are utilized during weaning. Thus, second litters, normally conceived following a post-partum estrus, are born when their mother's fat (energy) reserves have been used up. This typically leads to abandonment of the second litter. However, if a mother has lost her first litter to a predator or has abandoned it for other reasons (such as when resources are insufficient; Smith 1978; Smith and Ivins 1983), second litters appear to be a safety valve.

Litters are also small and average three (range 1–5) in American pikas. Due to the energetic constraints of weaning, there can be a gradual decrease of litter size due to pre-implantation losses, resorbtion of embryos, or losses during weaning. The end result is the production of approximately two young per mother per year (Millar 1973, 1974; Smith 1978). In contrast, Plateau pika mothers are baby machines, churning out fairly large litters (generally 4–8 young) at 3-week intervals during the reproductive season. Several litters (normally at least three) are produced each year, and most young are weaned successfully. The result is an increasing density within family burrow territories as the breeding season progresses, thus a high regional population density (Smith and Wang 1991; Dobson et al. 1998).

Social Dynamics

The solitary American pika is notoriously unsocial. I have spent hundreds of hours directly observing populations of American pikas that were all individually marked with colored ear tags. Observed social interactions are normally aggressive, with territory holders (male or female) chasing out intruding conspecifics (Smith 1981b, 1984; Smith and Ivins 1984). There are some “rules” however to these chases. Most pikas reside in territories adjoining an animal of the opposite gender, and chases occur only half of the time when these animals encounter one another. The same is true of their offspring—sometimes they are chased but at other times tolerated. On the other hand, animals that reside more than one territory away are nearly always chased. The talus and the pika territories are of sufficient size, however, that these chases occur on average only once in about 15 h of observation (Smith and Ivins 1984). There are further nuances in the social-spatial dynamics of American pikas; animals are more likely to intrude onto the territories of neighbors if the neighbor is not surface active at that time. This appears to be a tactic to avoid detection and the resultant long chase that may ensue (Smith and Ivins 1986; see Smith and Ivins 1987 for similar spatial temporal relationships between parents and offspring).

While no true social interactions can be observed between conspecific American pikas, there is one telltale behavior that characterizes the social system on a rock slope. About 95% of all instances when two pikas encounter one another and no chase ensues (a behavior category I have termed “social tolerance”), are between nearest neighbors of opposite gender (Smith and Ivins 1984). Similarly, nearly all occurrences of pikas duets are between male and female nearest neighbors (Smith and Ivins 1984). Thus, American pikas can and do discriminate among animals that may be a mating partner from others in the local population.

The social system of the Plateau pika could not be more different than that of the American pika. Plateau pikas are extremely social, and when the population density has peaked (following the weaning of a second or third litter), affiliative social interactions can be observed at the rate of one per minute (Smith et al. 1986; Smith and Wang 1991). These affiliative behaviors include allogrooming, sitting in contact, nose rubbing, and many other forms of contact. In addition, Plateau pikas utter up to six different vocalizations, many of which elicit these affiliative contact behaviors (Smith et al. 1986). Most of the interactions occur among juveniles of the same or different age, and many include an adult male (or putative father) on the family territory. Rate of expression of affiliative behavior by adult males is higher than for adult females. Over 95% of all affiliative behaviors observed in a population occur within family groups (Smith et al. 1986; Smith and Wang 1991; Dobson et al. 1998). In contrast, aggressive behaviors (long chases and fights) are normally expressed by animals from different family territories (normally these are adult male interactions; Smith et al. 1986; Smith and Wang 1991).

Dispersal and Population Structure

Intriguingly, one similarity between American and Plateau pikas (and indeed, all pika species that have been studied to date) is the degree to which individuals are sedentary. Stated another way, pikas are relatively poor dispersers. They are most likely to stay at or near their site of birth (remain philopatric), and dispersing animals normally do not range far from their natal territory. The American pikas I observed in the Rocky Mountains of Colorado rarely dispersed (Smith 1974b; Smith and Ivins 1983; Smith 1987). Over 85% of all adults that controlled access to a territory remained there for life. Of 45 juveniles born into our population over 3 years, only two dispersed (4%) within their home talus. Dispersal between talus patch habitats also appears to be an infrequent phenomenon (Tapper 1973; Smith 1987; Peacock 1997). Because American pikas are individually territorial and long-lived, openings (resulting from death of a territory resident) are rare and apparently normally claimed by animals born nearby who have the greatest familiarity with the socio-spatial structuring on the talus (Smith and Ivins 1983; Peacock and Smith 1997). One interesting consequence of the eventual settlement pattern of pikas into these vacancies is that they are normally claimed by animals of the same sex as the previous occupant and the apparent “locked in” structure of the population is normally an alternate male-female pattern (Smith and Ivins 1983, 1984). The only exception to this structure in the literature comes from a population that was a “dispersal sink” and had high mortality (thus multiple available sites) each year, so that neighboring animals could not control the eventual settlement pattern of dispersers (Brown et al. 1989; Brown, personal comm.).

Plateau pikas are also primarily philopatric (Smith and Wang 1991; Dobson et al. 1998). Few adults move to new family territories between years (because of the high mortality, few are available for such movements). Young remained in their family territories throughout their summer of birth and over winter. Over 57% of juveniles remained in their natal family territory as adults. Dispersal occurred just prior to the reproductive season in spring (Wang and Smith 1989), and males were more likely to disperse than females. Median dispersal distance of young males was two territories; females on average moved only one territory (essentially, next door; Dobson et al. 1998). In a multi-year investigation we detected no long-distance dispersal (Dobson et al. 1998).

Mating Systems

The basic mating system in both American and Plateau pikas is monogamy, although this result may be due to very different circumstances. American pika males largely associate with a single female because they cannot control multiple females (as females are widely dispersed across the talus), nor can they control enough habitat to gain access to multiple females. Thus, they are monogamous almost by default, a condition known as facultative monogamy (Smith and Ivins 1984).

On the other hand, Plateau pikas exhibit a range of mating system types in addition to monogamy; males may live in polygynous, polyandrous or polygynandrous families (Liang 1981; Wang and Smith 1989; Dobson et al. 2000; Smith and Dobson 2004). The proportion of mating systems of any given type varies among years. There is no relationship between habitat quality and mating system type; a family burrow system territory could include a polygynous system in 1 year and any of the other mating system types the next. Apparently the mating system established on any family territory is determined by the sex-ratio of overwintered animals modified by the relatively few dispersal movements just prior to the mating season (Smith and Dobson 2004).

Summary

This broad suite of characteristic differences between American and Plateau pikas (Table 1) represents an excellent opportunity to investigate how animals with similar appearance adapt to the very different habitats they occupy. The population dynamics, reproduction, behavior, dispersal characteristics or mating system dynamics have not been investigated in most species of pika. Much work remains to be done before we can have a comprehensive understanding of these traits across the genus *Ochotona*.

Table 1 Comparative characteristics of rock-dwelling and meadow/steppe-dwelling (burrowing) pikas

Characteristic	Rock-dwelling	Burrowing
Litter size	Small (1–6; generally 3)	Large (1–13)
Litters/year	1–2 (normally only one weaned)	3–5 (all weaned)
Reproductive maturity	Yearling	Young of the year
Longevity	Long-lived (up to 6–7 years)	Short-lived (normally 1+ years)
Density	Low (2–15/ha)	High (up to 300/ha)
Variation in density	Relatively constant	Highly Fluctuating
Mating system	Monogamy (facultative, or live in pairs)	Variable (monogamy, polygyny, polyandry)
Territoriality	Single or Pair	Family
Sociality	Asocial	Highly social
Behavioral repertoire	Limited	Complex
Vocal repertoire (if vocal)	Limited	Complex

American Pika Metapopulation Dynamics

Rock-dwelling pikas represent one of the best models for investigation of metapopulation dynamics. Metapopulations are interdependent patches of occupied and unoccupied habitat, thus are integral for describing the dynamics of fragmented populations. An ultimate goal of metapopulation research is to understand these systems sufficiently to be able to predict their behavior and manage for their persistence. Pikas are ideal for these studies because their obligate habitat type, rocks, is often found in discrete patches.

The population of American pikas at Bodie, California, presents a classic example of metapopulation dynamics (Smith 1974b, 1980; Peacock and Smith 1997; Smith and Gilpin 1997; Moilanen et al. 1998; but see Clinchy et al. 2002). At Bodie, small populations of pikas occupy mine tailing patches in a sea of sagebrush. At any one time, about half of the patches are vacant, although each patch is suitable habitat for pikas and we have evidence that all patches have at one time been occupied by pikas. I have studied the pikas at Bodie since 1969 and have conducted censuses of all patches annually for the past 12 years. Key variables in this system are the occupancy of patches (determined using a variety of features including direct sighting of territorial pikas and presence of fresh sign such as green haypiles, scat piles, etc.), patch size, and degree of isolation of each patch (distance to the nearest occupied patch, or patches).

Bodie pikas appear to be in a dynamic equilibrium between extinction of populations primarily on small habitat patches and recolonization from nearby patches (Smith 1974b, 1980; Smith and Gilpin 1997; Moilanen et al. 1998). This process is slow at Bodie because dispersal is so restricted, mainly due to the high temperatures faced by pikas during dispersal (Smith 1974a). Some years are marked with more frequent extinctions, some with more frequent recolonization episodes—but overall these tend to balance (Smith, unpublished data). More important, however, is that the pattern of patch occupancy at Bodie is highly autocorrelated; a neighborhood analysis indicates that most patches were strongly influenced by the average level of occupancy in surrounding patches. Thus, entire neighborhoods rather than distance to a single potential source patch appear most important in determining the probability of patch occupancy. When the number of nearby patches occupied falls below a threshold number, then entire regions may go extinct as has been observed in the southern network of patches at Bodie (Smith and Gilpin 1997). These results are chilling, in that they demonstrate the sensitivity of metapopulations to collapse. One take-home lesson is the challenge for conservation biologists to identify how to determine thresholds in sensitive species below which their metapopulation dynamics may become inherently unstable (Smith and Gilpin 1997).

There are many rock-dwelling pikas that could be studied to further our knowledge of metapopulation systems in other ecosystems. For example, Franken and Hik (2004) have used the metapopulation approach in their studies of the collared pika (*O. collaris*) to gain greater insight of the role of habitat quality on patch dynamics.

The Plateau Pika as a Keystone Species and Ecosystem Engineer

A keystone species is one whose loss from an ecosystem has a disproportionately large effect on other species, and species known as ecosystem engineers provide additional living space or resource opportunities to target species by altering the structure of their environment (Dickman 1999). The plateau pika is both a keystone species and an ecosystem engineer on the alpine meadow of the Qinghai-Xizang plateau (Smith and Foggin 1999; Lai and Smith 2003; Smith and Harris 2004). Plateau pikas:

- provide habitat for endemic species—we have shown that endemic birds such as Hume's groundpecker (*Pseudopodoces humilis*) and several species of snowfinch (*Montifringilla nivalis, Montifringilla adamsi, Pyrgilauda ruficolis, Pyrgilauda tacazanowskii, Pyrgilauda davidiana*) are most common on areas where pikas have not been poisoned; these species nest in pika burrows that collapse after pikas have been eradicated from a region);

- are a source of food for most predators including weasels (*Mustela* spp.), steppe polecats (*Mustela eversmanii*), Pallas' cat (*Felis manul*), snow leopard (*Uncia uncia*), foxes (*Vulpes* spp.), wolf (*Canis lupus*), brown bear (*Ursus arctos*), Saker falcon (*Falco cherrug*), upland buzzard (*Buteo hemilasius*), black-eared kite (*Milvus lineatus*), golden eagle (*Aquila chrysaetos*), goshawk (*Accipiter gentilis*), and little owl (*Athene noctua*);
- increase plant species richness due to disturbance, and
- increase ecosystem function properties (nutrient cycling, prevention of extensive erosion during the heavy monsoonal rains of summer).

These traits ensure maintenance of biodiversity on the plateau as well as providing benefits for human welfare. Unfortunately, plateau pikas have also been viewed as a pest species and up to 208,000 km^2 of the landscape occupied by pikas has been poisoned in an attempt to eradicate them (Fan et al. 1999). This control effort has been ongoing since 1958 and remains a central governmental activity for management of the increasingly degraded pastureland on the plateau.

Should the plateau pika be treated as a keystone species/ecosystem engineer or as a pest in efforts to manage the fragile alpine meadow ecosystem and ensure that its resources can be productive and sustainable for the benefits of Tibetan pastoralists? The answer to this question depends on (1) the extent that science is allowed to play a role in formulating environmental and development policy; (2) the development of thorough cost/benefit analyses of scenarios (long and short-term) that involve poisoning pikas or allowing them to persist, and; (3) a clear statement of objectives for effective management of the pastureland. Continuing work to understand more completely the ecological role of plateau pikas, in particular their contribution to ecosystem services, is underway in an attempt to shed more light on these issues.

Other pika species have been targeted as pests and poisoned: the Afghan pika (*O. rufescens*), Daurian pika (*O. dauurica*), and Pallas' pika (*O. pallasi*; Smith et al. 1990). In addition, there are many other burrowing meadow/steppe-dwelling pikas that potentially play similar ecological roles throughout their range (Komonen et al. 2003). It is important that studies be initiated on these forms to further our understanding of the overall ecological importance of the genus *Ochotona*.

Conservation Issues

Many pikas are highly endangered due to habitat loss, poisoning, climate change, stochastic metapopulation dynamics, and more. We need to identify those species that are threatened with extinction, to determine the causes, and to formulate a proactive conservation agenda to ensure their continued survival. Some current examples of threats to pika species (Chapman et al. 1990; Formozov 1997; IUCN 2005; IUCN Red List Criteria Ver 2.3 1994 in parentheses) include:

- Koslov's pika (*O. koslowi*) (EN B1 + 2abd): a steppe-dwelling species that is extremely rare and has a restricted geographic range that may be subject to encroaching agriculture and/or poisoning.
- Steppe pika (*Ochotona pusilla*) (VU A1cd, C2a): this species is vulnerable to habitat destruction. In historical times, the Steppe pika has retreated as its habitat was plowed under (Formozov, personal comm.).
- Hoffmann's pika (*Ochotona hoffmanni*) (VU D2), and the Silver pika (*Ochotona argentata*) (CR A2cd): these rock-dwelling forms are known only from extremely restricted areas and are vulnerable to chance extinction—particularly if any anthropogenic force further reduces their ranges or populations.
- Ili pika (*O. iliensis*) (VU D1+2): this cliff-dwelling species that is restricted to two branches of the Tian Shan mountains in China has declined precipitously in the past decade, and this decline may have been caused in part by the effects of global warming (Smith et al. 2004; Li and Smith 2005).

In addition, many subspecies of pikas have been identified as threatened (IUCN 2005). Beever et al. (2003) have determined that over a quarter of known isolated populations of the American pika in the intermountain west of North America have become extinct within the past few decades, a decline that they attribute in part to global warming. Almost all the habitat once occupied by the Indian population of the Moupin pika (*O. thibetana sikimaria*) has been destroyed, making this subspecies critically endangered. Due to the remote localities occupied by many pika species and populations, there are likely to be additional forms that are similarly threatened.

It is incumbent upon us to learn more of the current status of these interesting animals, to study them, and to determine accurately their correct systematic classification. I have outlined several avenues of exciting and important research using two pika species as my prime examples, but little is known about most of the other species. Pikas are also becoming increasingly well known and popular with ecotourists. The Pika Fan Club of Japan, for example, uses the Northern pika (*O. hyperborea*) as a model for ecotourism, and this approach has led to the protection of large tracts of land that had been targeted for development (Ichikawa 1999). I hope that there will be a resurgence of interest in pikas along all of these dimensions, and that at the next World Lagomorph Conference we can look forward to a balanced representation of studies on these wonderful animals.

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When? Where? and for How Long? Census Design Considerations for an Alpine Lagomorph, the Collared Pika (*Ochotona collaris*)

Shawn F. Morrison* and David S. Hik

Introduction

Talus-dwelling pikas (*Ochotona* spp.) live in alpine areas on naturally fragmented patches of talus habitat separated by an inhospitable matrix of meadow or forest. Consequently, pikas have been studied to examine dispersal behavior (Peacock and Smith 1997) and to test predictions of metapopulation theory (Clinchy et al. 2002; Moilanen et al. 1998; Smith 1980). Their small territories, diurnal behavior, and high levels of activity have also made them the focus of studies investigating foraging behavior (Dearing 1996; Holmes 1991; Morrison et al. 2004), nutrient cycling (Aho et al. 1998), and plant community composition (Huntly 1987; Mcintire and Hik 2002), among others.

Pikas have been recognized as being particularly vulnerable to the effects of climate warming because of their sensitivity to high temperatures (MacArthur and Wang 1973; Smith 1974). This sensitivity had led to pikas being proposed as climate-change indicators for alpine ecosystems (Beever et al. 2003; McDonald and Brown 1992; Smith et al. 2004), in part because alpine and high latitude ecosystems are predicted to be most affected by global warming (Källén et al. 2001). Some negative effects of climate change on high-latitude wildlife populations are already being observed (Derocher et al. 2004; Hik 2001), however the overall implications of climate warming remain unknown for most species (Hofgaard et al. 1999).

Testing ecological theory in the field and managing wildlife populations and their habitat requires reliable estimates of population density among sites and over time, and both direct enumeration techniques and population indices are widely used (Caughley and Sinclair 1994; Karels et al. 2004; McArdle et al. 1990; Wilson et al. 1996). Indeed, for pikas to be useful as an indicator species, long-term population census data are essential for detecting changes in pika abundance, population growth rates, and the range of natural variation. Long-term data will also permit accurate parameterization of population models for determining the future viability of pikas at local, regional and population scales (e.g., Beissinger and McCullough 2002).

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This, in turn, will allow for an assessment of the alpine ecosystem as a whole as reflected by pika populations. Unfortunately, there are few long-term population census datasets available for this genus.

In this study, we used data from a continuous 10-year (1995–2004) live-trapping census for collared pikas (*Ochotona collaris*) to examine the effects of (1) census duration, (2) location of the study area, and (3) timing of study initiation on estimates of population size and the yearly rate of population growth (λ). Our primary objective was to determine the number of years of census data required to quantify the population dynamics of this species.

Study Area

The study was conducted in a 4-km^2 alpine valley that consisted of a meadow interspersed with patches of talus in the Ruby Range, east of Kluane Lake, Yukon, Canada (61°13′N, 138°16′W; 1,700–2,200 a.s.l.) between 1995 and 2004. The talus patches were separated by a matrix of *Dryas octopetala*, *Salix* spp. and several graminoid species (e.g., *Carex consimilis*). *Cassiope tetragona* was common along the talus margins in some areas. See McIntire (1999) and Hik et al. (2001) for additional details.

The valley was segregated into three subpopulations based on dominant aspects: east-, west-, and south-facing. Collared pikas, hoary marmots (*Marmota caligata*) and arctic ground squirrels (*Spermophilus parryii plesius*) were the dominant herbivores in the valley. Potential predators of pikas include raptors, foxes (*Vulpes vulpes*), and weasels (predominately short-tailed weasels, *Mustela erminea*) (Hik et al. 2001). The snow-free season generally extends from mid-June to early September.

Methods

We used a 10-year census dataset of >400 collared pikas where the entire population was uniquely marked and trapped each summer. Capture methods were described in detail by Franken and Hik (2004). Briefly, pikas were live-trapped using Tomahawk live-traps baited with fresh native vegetation. Pikas are quite active within their small (<25-m radius) territories, have distinctive territorial calls, and have distinguishable haypiles (Smith 1974, 1980), permitting us to locate and capture all pikas resident within the study area.

Individuals trapped for the first time were marked with numbered metal ear tags (Monel #1) and a unique color combination of thin wire to allow identification from a distance without requiring subsequent recaptures. Individuals were sexed following Duke (1951) and classified as juvenile or adult based on mass and molt pattern.

Bootstrapped mean population sizes and population growth rates ($\lambda_t = N_{t+1}/N_t$, where N_t is the number of pikas in year t) (Efron 1982) were calculated for 2–10 years of consecutive data for as many study-durations as possible for each subpopulation. For example, we had 7 years of consecutive census data available for the south-facing subpopulation (1998–2004), which allowed us to calculate bootstrapped means for six possible 2-year studies (1998–1999, 1999–2000, . . . , 2003–2004), five 3-year studies, four 4-year studies, and so on. Coefficients of variation (CV; Zar 1999) were used to indicate variability in the mean values. In the above example, the CV for studies of 2-year duration was based on six bootstrapped means. We present examples of state variables (N_t) and transition probabilities (λ_t); similar analyses could be conducted on other population variables such as survival or reproduction.

Results

Population estimates and growth rates varied considerably and depended on study duration, year of initiation, and location within the valley (subpopulation). All three subpopulations were variable from 1995 to 2004 (Fig. 1). The east-facing and west-facing subpopulation declined from 1995 to 2004, with short periods of moderate increase (e.g., 2000–2002). The west-facing subpopulation declined to extinction in winter 1999–2000 and was not recolonized until 2001. The east-facing subpopulation went extinct during winter 2003–2004 but was recolonized by juveniles during summer 2004. The south-facing

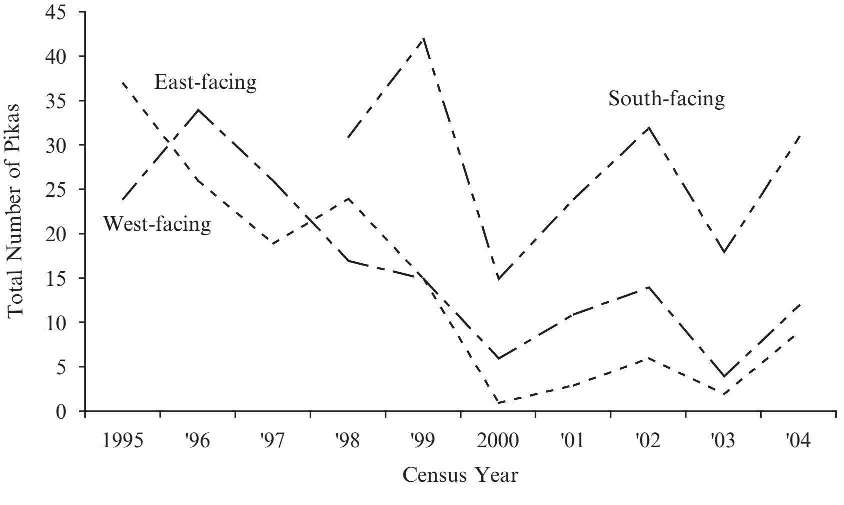

Fig. 1 Late summer total abundance of collared pikas (adults and juveniles) in southwestern Yukon from 1995 to 2004. Estimates were derived from complete census data of live-trapped individuals in three subpopulations

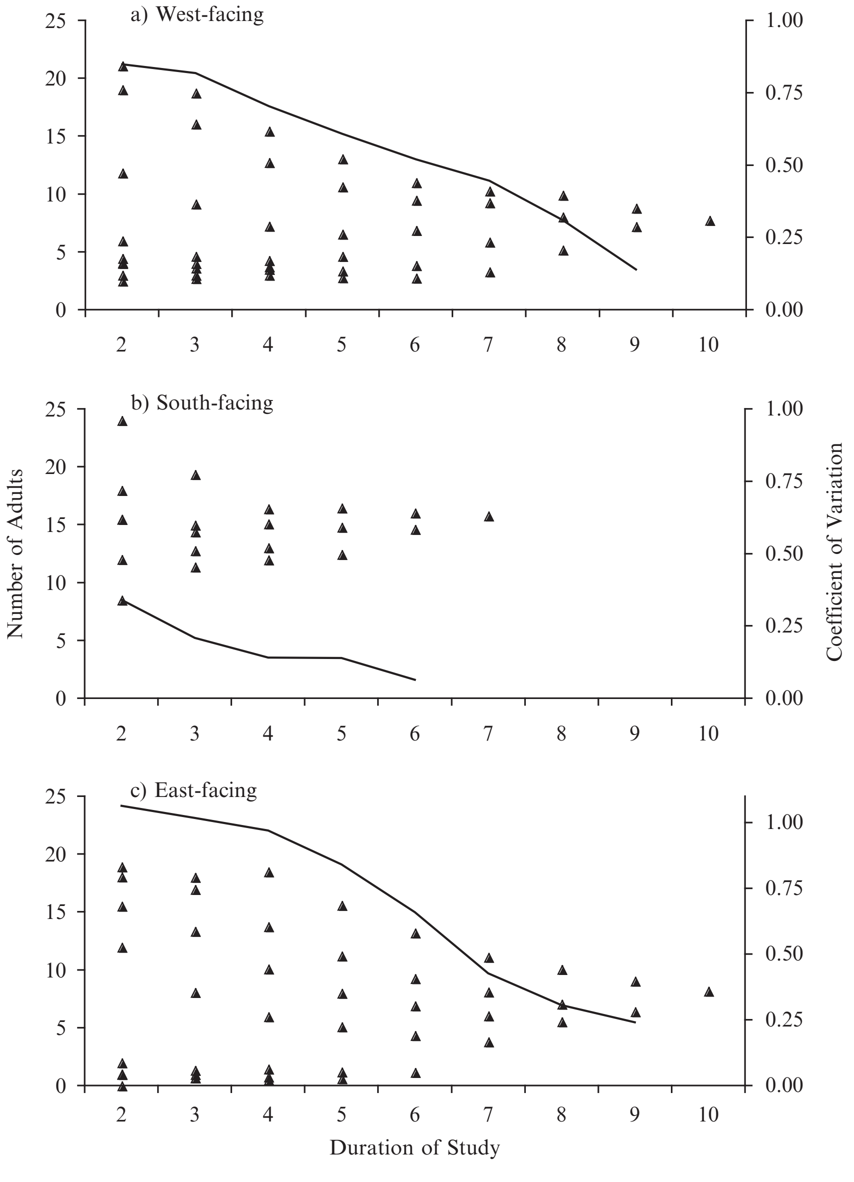

Fig. 2 Bootstrapped estimates of adult pika abundance using 2–10 years of census data. *Triangular symbols* represent bootstrapped mean number of adults in three subpopulations for hypothetical studies of 2–10 years in duration. The *solid line* indicates the coefficient of variation

subpopulation was consistently larger than either the east-facing or west-facing subpopulations and did not go extinct at any time during our study (Fig. 1). Based on trapping data, only 4 of 400 pikas moved between these three subpopulations from 1995–2004 following their first capture, suggesting these areas were largely independent despite being <300 m apart.

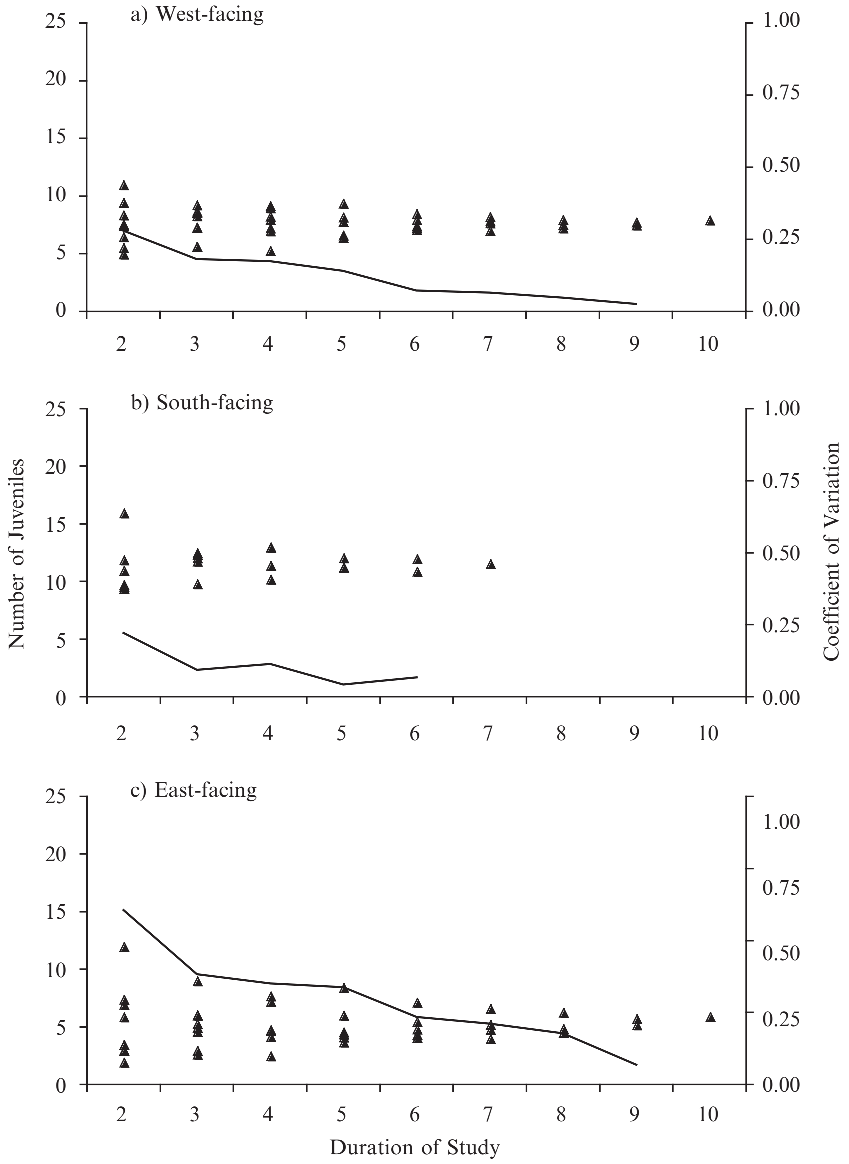

Fig. 3 Bootstrapped estimates of juvenile pika abundance using 2–10 years of census data. *Triangular symbols* represent bootstrapped mean number of juveniles in three subpopulations for hypothetical studies of 2–10 years in duration. The *solid line* indicates the coefficient of variation

The coefficients of variation (CVs) for population size were lowest for the south-facing subpopulation for all possible study durations. They (CVs) were remarkably small (<25%) for 2-year studies (Figs. 2–4), in marked contrast to the east-facing or west-facing subpopulations that required 5–6 years of data before CV declined to 25%. The CV continued to decline with additional

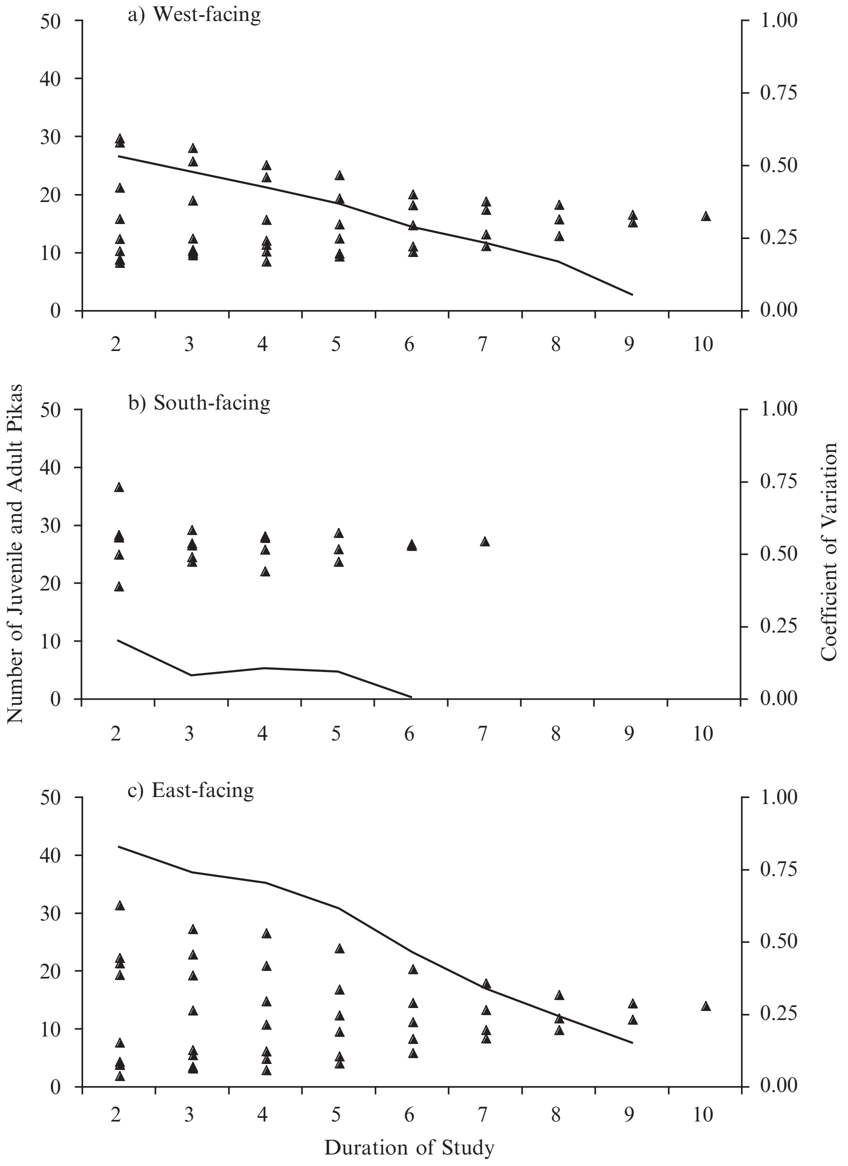

Fig. 4 Bootstrapped estimates of juvenile and adult pika abundance using 2–10 years of census data. *Triangular symbols* represent bootstrapped mean number of individuals (juveniles + adults) in three subpopulations for hypothetical studies of 2–10 years in duration. The *solid line* indicates the coefficient of variation

years of continuous data for east-facing and west-facing suggesting, not surprisingly, that a greater number of years were necessary to reliably estimate mean population size and variability.

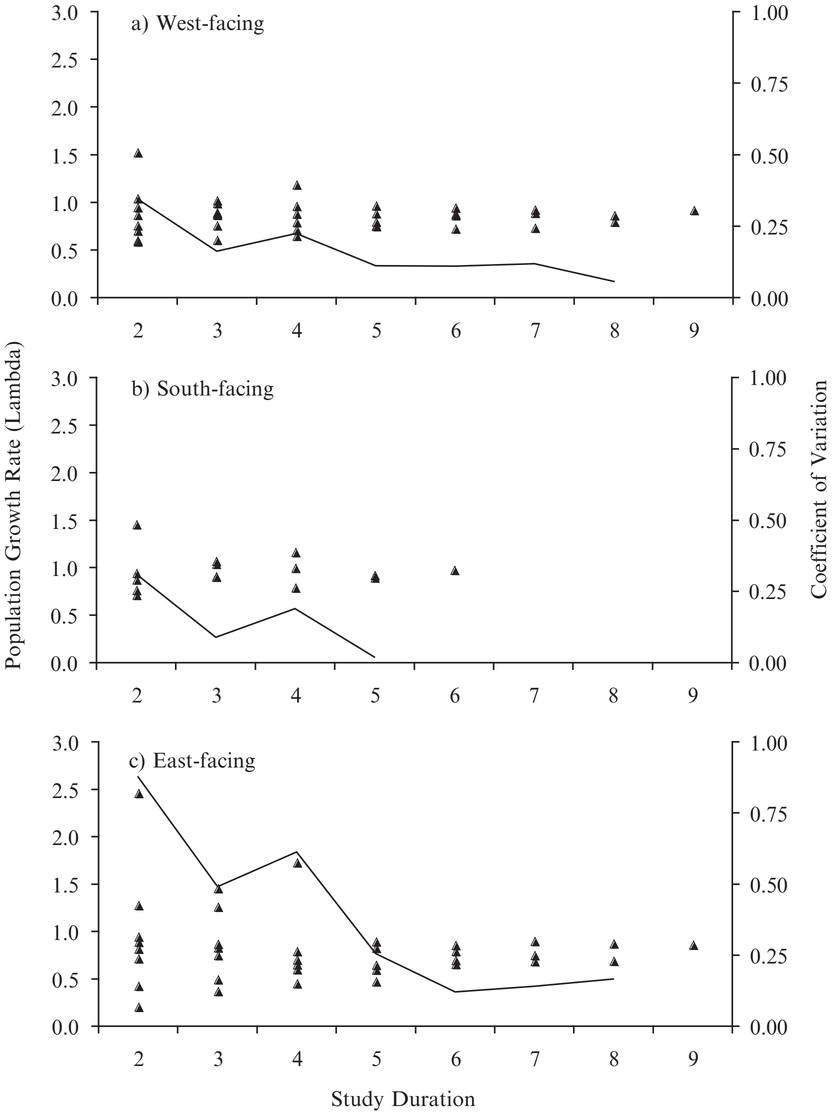

Fig. 5 Bootstrapped estimates of pika population growth rates using 2–10 years of census data. *Triangular symbols* represent bootstrapped mean growth rate in three subpopulations for hypothetical studies of 2–10 years in duration. The *solid line* indicates the coefficient of variation

The CVs for yearly subpopulation growth rates (Fig. 5) were lowest for the south- and east-facing subpopulations for all possible study durations, and declined to <25% with 3 years or more of census data. This result is in marked contrast to the east-facing subpopulation that required 5–6 years of data before the CV declined to less than 25%. The CV continued to decline with additional years of continuous census data for all areas suggesting again and, not surprisingly, a greater number of years were necessary to reliably estimate mean population size and variability.

Discussion

Collared pika populations in Yukon are variable across time and space, presumably in response to unpredictable environmental conditions (Smith 1978). This explanation for high variability may apply to our population that collapsed by 90% from 1998 to 2000. The decline occurred across the entire study area and does not appear to be related to biotic factors such as disease, food resources, or predation. Instead, we hypothesize that the decline is related to warmer winters that resulted in low snow accumulation (and therefore poor insulation value), increased frequency of freeze-thaw events, icing following winter rains, and late winter snowfall which delays the start of the growing season (Kreuzer and Huntly 2003; Smith 1978). If this hypothesis is correct, we predict that pikas at our study area will experience future population declines as a result of increased frequency of these adverse weather events as predicted by current climate change models (Houghton et al. 2001). In addition, climate warming is predicted to force pika populations to higher altitudes to maintain thermoregulation and could cause localized extinctions, particularly for lower altitude populations (McDonald and Brown 1992).

Population models created to understand pika population dynamics will require reliable parameter estimates (Beissinger 2002). This is particularly important when pikas are being used as a climate-change indicator species and are used to gauge the state of the surrounding alpine ecosystem. Further, accurate parameter estimates are essential when investigating the effects of stochastic environmental events (Lande 2002), that are predicted to increase in frequency by current climate models (Houghton et al. 2001).

Our study underscores the usefulness of long-term population monitoring. Although we presented 10 years of continuous census data, this may still not be of sufficient duration to determine the full range of natural population variability. Population size was relatively high in 1995 when the study was initiated but has declined since then with no indication of a recovery to previous numbers. This parallels declining trends reported for other species of pika such as *Ochotona princeps* (Beever et al. 2003) and *Ochotona iliensis* (Smith et al. 2004). There is no evidence to suggest that the high densities in 1995, or the low densities in 2000, are typical or anomalous. The maximum number of pikas is limited by territory availability, however, not all known territories, as indicated by old haypile remains, were occupied at the same time (DS Hik et al. unpublished data).

The low levels of observed movement between the three dominant aspects in our study site are consistent with other reports of pika philopatry

(McDonald and Brown 1992; Smith and Ivins 1983). Inter-patch movement rates of marked juveniles within a given aspect are higher than between aspects, as expected for individuals searching for vacant territories (Franken 2002). However, movement rates between patches and dominant aspects may be underestimated because juveniles are most trappable once they have established territories and are no longer moving between patches. Nevertheless, subpopulations were separated by non-talus habitat (streams and 100 to 200-m stretches of meadow), and these features likely serve as movement barriers to dispersing pikas, which are known to have poor dispersal abilities (Hafner and Sullivan 1995; Smith 1974).

Our results show that the year in which a study is initiated, its duration, and the sampling location within relatively small study areas are of great importance in inferring long-term population means and natural levels of variability. Conclusions based on short-term studies (<5 years) regarding longer-term population dynamics should be tentative, and extrapolation to nearby populations is cautioned. Given a choice, we recommend future monitoring studies give priority to sampling from multiple populations rather than collecting exhaustive census data on a single population. Population estimates could be obtained quickly and relatively inexpensively by searching for active hay piles in late summer as an indication of pika presence. These data could then be incorporated into a count-based population viability analysis (PVA) to determine extinction risk (Morris and Doak 2002). Alternatively, and given additional time or funding, live-trapping and ear-tagging should be added to provide useful information on age structure and survival. This would allow for the construction of detailed demographic PVAs that more accurately portrays population dynamics than count-based methods (Morris and Doak 2002).

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Weather Effects on Reproduction, Survival, and Body Mass of European Rabbits in a Temperate Zone Habitat

HEIKO G. RÖDEL* AND DIETRICH VON HOLST

Introduction

Understanding the effects of environmental factors on the dynamics of animal populations is one of the major challenges for population biologists. In particular, the impact of climate and weather has been the focus of many studies during the last years (e.g., Lima and Jaksic 1998; Milner et al. 1999; Stenseth 1999; Yoccoz and Stenseth 2000; Aars and Ims 2002; Lande et al. 2003). However, data on specific effects of the weather on different vital rates are rare, because, in small mammals in particular, individually based studies are difficult to conduct, and long-term datasets are needed for obtaining reliable results.

The European rabbit *Oryctolagus cuniculus* is an interesting and very suitable model organism for such a study. Formerly restricted to a relict distribution on the Iberian Peninsula about 3,000 years ago, this species has been spread by man over nearly all continents and to different climatic zones (Flux 1994). This species is characterized by a high reproductive capacity (e.g., Myers and Poole 1962; Gonçalves et al. 2002; von Holst et al. 2002) but also by high mortality rates, in particular in juveniles (e.g., Richardson and Wood 1982). This can result in strong short-term fluctuations in population numbers (e.g., Erlinge et al. 1984; Myers and Poole 1963). Furthermore, several studies on European rabbit populations point out, that climatic and weather conditions have a strong impact on the abundance and on population densities of this small mammal (e.g., Erlinge et al. 1984; Trout et al. 2000; Schröpfer et al. 2000; Calvete et al. 2004).

Since 1988 we have kept and studied a population of European rabbits in a field enclosure of 2 ha in Upper Franconia, Germany. In this chapter, we review our findings about the effects of weather on different reproductive components, on the body mass of juveniles at the end of the vegetation period, and the over-winter survival of adults and first-year individuals' reproduction, survival, and body mass. Many aspects presented here have been described in our previous papers, however not all results have been published.

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Our Study Population in Bayreuth, Germany

Our study population consisted of animals that were descendants of individuals that had been caught at different sites in south Germany. Vegetation in the 2-ha enclosure consisted of homogeneous grassland with interspersed groups of trees and bushes. The vegetation growing in the enclosure was the only food source for the rabbits during the vegetation period. Nevertheless, we provided restricted amounts of hay in ten feeding racks during periods of harsh winter conditions (mostly occurring in late January), when high snow cover or snow crusts prevented the rabbits' access to ground vegetation. The animals in our study population were hosts to several species of endoparasites (Eimeria, Trichostrongylidae, Oxyuridae) as are all rabbits living in the wild, but we had no cases of myxomatosis or rabbit hemorrhagic disease during the study period from 1991 to 2002. The access of most of the common predators (e.g., *Martes foina, Mustela erminea*, *Mustela nivalis*, *Accipiter gentilis*, *Buteo buteo*) was not restricted by the fence. In our study population, the animals never reproduced within their year of birth. However, first-year animals, at least the ones which were born early in the season, have reached physiological maturity by autumn, and are therefore hereafter referred to as 'subadults'.

Methods and Data Collection

Apart from a daily walk to check the enclosure in the morning and a monthly trapping session, there was no other human interference. The entire study site could be observed from two separate outlook towers and all the animals could be identified by their individual ear-tags (colored aluminum tags fixed with Dalton Rototag, 35 × 10 × 2 mm). In addition to the burrows dug by the rabbits, the area contained 16 artificial concrete warrens with interconnecting chambers and removable covers, which were evenly distributed throughout the study area. These were used by the rabbits as the main warrens of their group territories and also for breeding.

Once a month, the animals were caught by the aid of peanut-baited wooden traps set overnight (in summer) or in the morning for several hours (in winter) and the captured animals were stored separately in sacks and later weighed.

We checked for newborn litters every morning during the breeding season. In order to do this, we prepared burrow systems and breeding stops dug by the animals with artificial holes, which we covered with concrete flagstones. At postnatal day 12, we weighed all pups of each litter and marked them individually using colored ear tags (Dalton Rototag, 20 × 5 × 1 mm). We regularly dyed the ventral fur of all adult females of each group with different colors (Marabu silk color). Female rabbits pluck out the hair from their ventral fur in order to build their nests, which enabled us to determine the maternity of each litter by the color of hair found in the nest. We additionally validated this by the behavior and space use of the females in relation to the location of the breeding site. Data on space use, social behavior, group membership, and digging of breeding sites of each adult female were collected by regular direct observations throughout every breeding season. This was done during the last 3–4 h before dawn when the rabbits showed their main social activity (Wallage-Drees 1989). We determined over-winter survival of all subadults that were known to be present in autumn by counting the surviving animals in early March. Thus, each individual studied could be assigned to the category 'survivor' or 'non-survivor'. All weather data were provided by a meteorological station which was situated 400 m away from the enclosure.

Results and Discussion

Reproduction

The results of our study suggest that the timing of the breeding season of the European rabbit in the temperate zones is modified by at least two different weather variables. Firstly, the onset of breeding was related to the temperature conditions of the previous winter season (Fig. 1; cf. Rödel et al. 2005). Secondly, we found that the decrease in the seasonal reproductive activity in summer was correlated with the amount of precipitation in summer (Fig. 2b). The latter finding has not been published by us elsewhere.

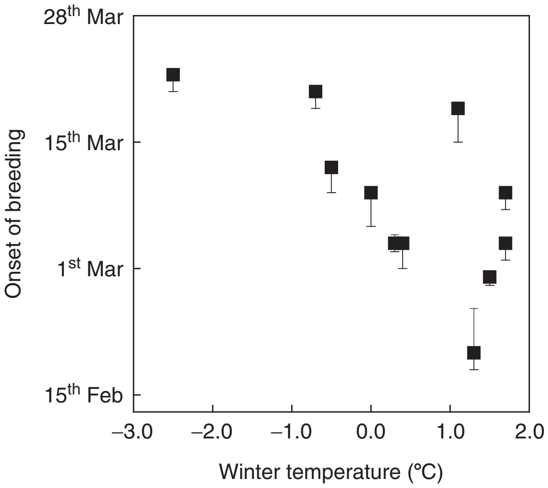

Fig. 1 Correlation between the average winter temperature and the annual onset of the breeding season ($r_s = -0.661$, $n_{\text{years}} = 11$, $P = 0.027$), measured as the median parturition date (plotted with 25, 75% percentiles) of the first 25% of the female population less 30 days of gestation (modified from Rödel et al. 2005)

The fact that European rabbits in the temperate zones shift the onset of the breeding season in response to harsh winter conditions has been already described for rabbit populations in southern Sweden (Anderson et al. 1979) and England (Bell and Webb 1991). In addition, the results of our long-term study underline the strong impact of this weather factor: the maximum range in the onset of breeding between the years studied was 33 days, which approximates to the length of the gestation period of this species (30–32 days: Hudson et al. 1995). Similar effects of winter weather have also been reported for other seasonal breeding lagomorph species (e.g., *Sylvilagus floridanus* (Eastern cottontail): Hamilton 1940; Wright and Conaway 1961; *Lepus townsendii* (White-tailed jackrabbit): Kline 1963; *Lepus europaeus* (European hare): Flux 1967), whereas this effect may be primarily based on the lower pre-breeding body condition of the females due to a reduced food availability or quality during harsh winters. However, in contrast to the findings of Bell and Webb (1991), our study did not support a reduction in fecundity or reproductive performance in response to harsh winter weather conditions (Table 1). Our data rather suggest that the females compensated their lower body condition after harsh winters, which might potentially affect their reproductive performance by the observed delay in the onset of breeding (see Rödel et al. 2005). In contrast, we found a negative correlation between the winter temperature and the average fecundity during the subsequent breeding season (see Table 1). We speculate that this unexpected relationship was caused by density-dependent reproductive suppression due to the higher population number, in particular the number of recruits, after mild winters (cf. Myers and Poole 1962; Rödel et al. 2004a, 2004b).

The females of our population showed a strict seasonality in reproduction. During our 11-year study period, no littering was observed from November to February. Every year, the females reproduced at least until autumn (unpublished data). The reproductive activity, which we measured as the

Table 1 Spearman correlation between winter temperature and different measures of reproductive performance/fecundity during the subsequent breeding season, averaged over the annual female population ($n_{\text{years}} = 11$). Litter size, litter mass, and litter mass gain during the first 12 days of lactation were tested for litters of the first reproductive cycle/season. Data were average over 16–31 females per season. Only females older than 1 year were included in the analysis, since 1-year-old females were known to have a consistently lower reproductive performance (modified from Rödel et al. 2005)

Response variable	r_s	P
Offspring per season	–0.770	0.006
Litters per season	–0.633	0.036
Litter size	–0.410	0.210
Litter mass	–0.446	0.169
Litter mass gain (day 1–12)	–0.396	0.228

average proportion of reproducing females/months, peaked in May and then sharply declined to July/August (Fig. 2a). Studies in warm and arid regions point out that the availability of green pasture in response to rainfall restricts the reproductive activity of the rabbit during or at the end of the breeding season (Mykytowycz 1958; Soriguer and Rogers 1981; King et al. 1983; Wheeler and King 1985). Even if green pasture was area-wide available until October in all of the years and also the access to water was never restricted in our study population, we also found strong support for this effect: The decrease in the reproductive activity from June to July was significantly correlated with the amount of rain at this period of time (Fig. 2b).

Body Mass of the Juveniles at the End of the Vegetation Period

In seasonal environments, the survival of juveniles or first-year individuals of many mammal species is strongly affected by the body mass that the animals reach until the end of the vegetation period (Marboutin and Hansen 1998; Loison et al. 1999; Rödel et al. 2004a). For European rabbit populations in arid habitats, the restriction of green pasture by low precipitation is certainly one of the key factors for limited growth rates and therefore low autumn body masses of juveniles, whereas this effect might be enhanced by a high population density (e.g., Dudzinski and Mykytowycz 1960). However, in our temperate zone population, we found a strong negative correlation between rainfall during the early juvenile period and the autumn body mass (Fig. 2; see also Rödel et al. 2004a). Young rabbits that were exposed to a higher proportion of rainy days during the first 2 months after first emergence above ground had a lower body mass in mid November. We suggest two different mechanisms which may have caused the limited growth rates in individuals that experienced rainy weather

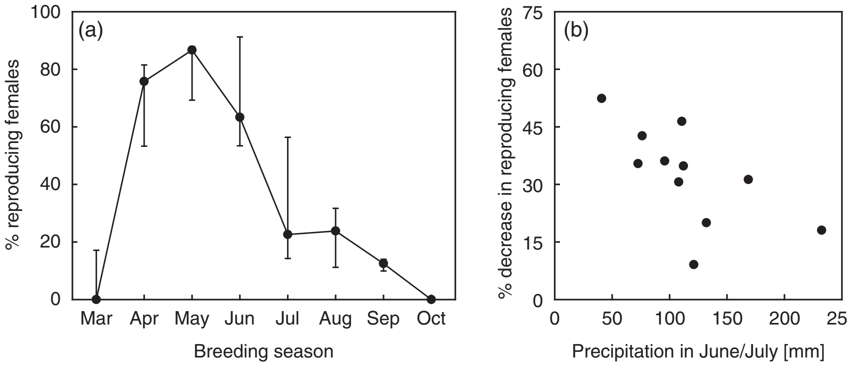

Fig. 2 a Proportion of reproducing females on the total female population per breeding season (median with 25, 75% percentiles), and **b** correlation between the amount of rain in June/July (measured from June 15–July 30) and the annual decrease in the percentage of reproducing females from June to July ($r_s = -0.755$, $n_{\text{years}} = 11$, $P = 0.007$)

conditions during their early development: Firstly, the heat loss, and secondly the risk of infections from diseases, which may both increase when the young rabbits are permanently wet, might negatively affect the energy allocation for growth. In particular, the infestation with coccidiosis, which is latently present in almost all European rabbit populations (e.g., Mykytowycz 1962; Stodart 1968; Cowan 1985), may play a major role, since the extracorporeal persistence of the infective sporocysts is prolonged in humid environments.

Winter Survival

Over-winter survival of subadult rabbits was significantly influenced by two weather variables: the precipitation that the animals were exposed to during their early juvenile period after emergence above ground, and the winter temperature (Fig. 3). Cold winters and frequent rain during the early juvenile period lowered the chance of winter survival. Subadults born early in the season had a higher chance of survival, whereas survival was decreased at high population density. Furthermore, the survival in females was slightly higher than in males. In our original paper (Rödel et al. 2004a), we tested various interactions among these candidate factors and also tested the support of simpler models with a lower number of parameters. However, model selection by Akaike's information criterion revealed that the model including the additive

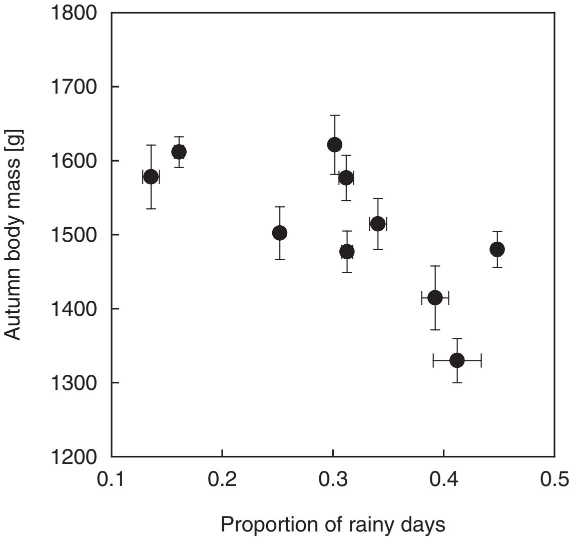

Fig. 3 Correlation between the proportion of rainy days (=days with precipitation >1 mm) to which the individual juveniles were exposed during the first 2 months after emergence above ground at around day 20 after birth (averaged per year, ± SD) and the mean autumn body mass measured in mid November (± SD; $r_s = -0.745$, $n_{\text{years}} = 10$, $P = 0.013$). Only data of juveniles born during the first reproductive cycles are included in the analysis (modified from Rödel et al. 2004a)

combination of winter weather, precipitation during early development, population density, date of birth and sex had the best support in explained over-winter survival of subadults.

This limiting effect of winter weather agrees with the findings on other European rabbit populations in temperate zones. Erlinge et al. (1984) noted a population breakdown in southern Sweden after harsh winter conditions, and Wallage-Drees (1986) reported an increased mortality in first-year animals during a winter with heavy snowfall. The higher costs of thermoregulation and the increased thermal stress during harsh winter conditions (e.g., Myers et al. 1977; Katzner 1997) together with the restricted access to high-quality food (Crawley 1983) may be the main reasons for the limiting effects of winter conditions on survival. Therefore, a high body mass, which has been found to be related to a high body fat content in the European rabbit (Wallage-Drees 1986), may substantially increase the chance of winter survival. In turn, the rainy weather conditions after first emergence above ground have been shown to affect the body mass of the subadults in autumn, which explains the observed impact of this factor on the probability on over-winter survival (Rödel et al. 2004a). Furthermore, a higher parasite load of individuals that experienced higher amounts of rain during their juvenile period may also translate into a lower chance of winter survival (Fig. 4).

In contrast to the strong effects of weather conditions on over-winter survival of subadults, preliminary analyses of our data did not support a relationship between winter weather and adult survival, or at least we did not find any significant correlations between the average temperature during the winter season and the survival rates of adults of both sexes (adult males: $r_s = 0.047$, $n_{\text{years}} = 15$, $P = 0.869$; adult females: $r_s = 0.100$, $n_{\text{years}} = 15$, $P = 0.722$).

Differences between these two age classes were also apparent in our study with regard to winter body mass loss. A study over 1 year revealed that subadults, which survived until the end of the winter season, lost on average 22.3%, whereas adults only lost 9.3% of their autumn body mass (Rödel 2000; Rödel et al. 2005). There are several reasons why young, first-season individuals might react more sensitively to adverse environmental conditions. Adult rabbits have a higher body mass compared to individuals short before reaching maturity: subadults of our study population that were born during the first reproductive cycle, and therefore had an average age of about 7–8 months in mid November, were still about 15% lighter that adults (unpublished data). Furthermore, juveniles or subadults generally obtain a lower social status, which may result in lower access to limited resources important for survival, such as the access to the main warren or high-quality feeding sites. Moreover, young rabbits are frequently chased out of the group territories by resident adults, in particular in autumn and early winter (Myers and Poole 1961; Cowan 1987; von Holst 1998). This behavior might generally cause negative stress effects and increase the energetic costs in this age class.

In conclusion, our studies reveal that different weather factors act together in limiting vital rates of European rabbits in the temperate zones. Firstly,

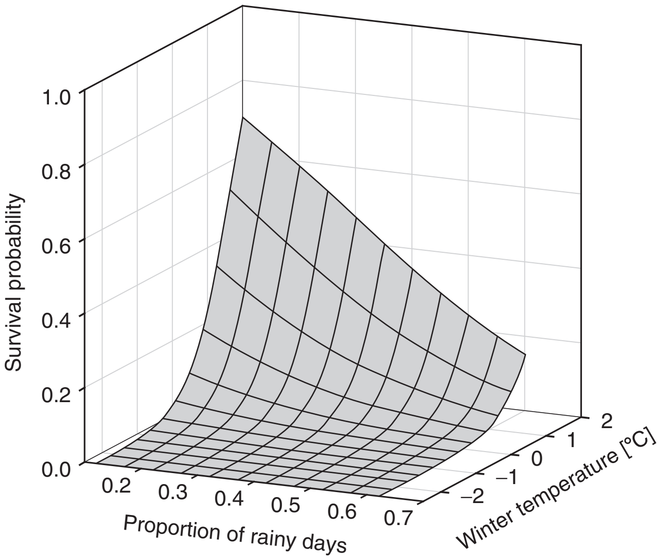

Fig. 4 Model graph for the probability of over-winter survival S of subadults in relation to winter temperature t and the proportion of rainy days p (=days with precipitation >1 mm) to which the individual juveniles were exposed during the first 2 months after emergence above ground. The logistic model is based on the data of winter survival of 657 individuals from 10 years, see statistics in Rödel et al. (2004a). Estimates were based on the logistic regression model $S = 1/(1+e^{(4.84 + 2.07t - 0.056d - 0.016b - 5.20r - 0.259s)})$, where s represents sex ($s_{\text{male}} = 0.5$, $s_{\text{female}} = -0.5$). Only exemplary data of female subadults are shown. The graph for males follows the same run, however on a lower level. The effects of population density d and date of birth b were set constant at their means

the onset and the regression of reproductive activity were affected by winter temperature and summer rain, respectively. Secondly, over-winter survival of first-year animals was directly affected by winter temperature and by the indirect action of rain in spring and early summer on the animal's pre-winter body mass.

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Ecosystem Engineering Effects of European Rabbits in a Mediterranean Habitat

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Introduction

Beyond their role as primary consumers, herbivore activities can play a key part in spatial processes at the ecosystem level (e.g., McNaughton 1983; McInnes et al. 1992; De Miguel et al. 1997). Environmental factors such as geomorphology, soil and vegetation characteristics, slope, aspect etc., affect the spatial distribution of the resources they need, mainly refuge, food, and water. Therefore, a particular set of habitat characteristics influences the use of different areas for specific activities by a given herbivore species. In turn, the use of habitats by herbivores may affect such characteristics at different scales. This is the case of many sedentary herbivores which, after establishing themselves in a particular area of an ecosystem, begin their modification. Pond construction by beavers not only changes river-flow patterns and surrounding vegetation, but also nutrient cycling (Naiman et al. 1994; Pollock et al. 1995). Excavating mammals, such as prairie dogs and pocket gophers, can also have profound impacts upon soil processes and the vegetation surrounding their burrow systems (see Whitford and Kay 1999; Huntly and Reichman 1994 for thorough reviews). Species that can have significant effects on the spatial structure of the landscape and the distribution of resources, both for themselves and other organisms, are considered ecosystem engineers (Jones et al. 1994). These species can be important patch creators through their activities or structures, and these patches can be exploited by different animal and plant species, thus increasing biodiversity and potentially controlling many processes and affecting ecosystems at different scales.

The European rabbit (*Oryctolagus cuniculus* L.) belongs to this category of sedentary herbivores and is unique among lagomorphs in its social and excavatory behavior. Their burrow systems (or warrens) can reach high densities and may have a large extension (radius of more than 15 m), with effects detectable even farther away (Gillham 1955; Lange and Graham 1983). Rabbit

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activities include excavating, trampling, pellet deposition, browsing, etc. These activities are frequently materialized in defined structures such as burrows, associated mounds, scrapes, latrines and paths, which generate patches with different characteristics. The area surrounding warrens is subjected to high levels of such disturbance, which affects mainly soils and vegetation (Eldridge and Myers 2001). These effects follow a distinct gradient, determined by rabbit use (Gillham 1955). Latrines are clearly defined areas of pellet accumulation and soil perturbation, used as territorial beacons (Cowan 1987; Sneddon 1991). They are potentially the most important structure from the vegetation point of view, as they induce physical and chemical changes on soil properties. In Mediterranean semi-arid regions, they are important contributors to soil fertility (Willot et al. 2000) and increase plant diversity and biomass (Petterson 2001). Latrines have also been shown to facilitate plant establishment in disused quarry soils in more mesic environments (Hambler et al. 1995). This ecosystem engineer role of rabbits has received little attention in ecological studies carried out in their native habitats of the Iberian Peninsula, where the long coexistence with other animal and plant species could have conditioned the dynamics of these Mediterranean ecosystems more than previously thought. Therefore, the European rabbit can be classified as a keystone species of this ecosystem.

The main aim of this chapter is to describe rabbit engineering effects on an Iberian Mediterranean ecosystem. For this, we explored the environmental factors affecting warren location in the landscape and analyzed the effects that two structures created by rabbits, warrens and latrines, have on the herbaceous community within rabbit home ranges. We expected different preferences for warren building according to geomorphology, vegetation characteristics, and management practices. We also hypothesized that rabbit structures would promote changes in the herbaceous community around warrens, and thus be an additional source of heterogeneity in dehesa ecosystems.

Materials and Methods

Study Site

'Dehesas' (in Spain) or 'montados' (in Portugal) are man-made savannahs from former oak woodlands that support the highest rabbit densities in the Iberian Peninsula. The present study was carried out in central Spain on a 225-ha dehesa situated in Chapinería, south-west of Madrid (40°23′ N, 4°12′ W). The mean altitude is 670 m a.s.l., and the climate is continental Mediterranean, with 12°C and 432.6 mm of mean annual temperature and precipitation, respectively. The substrate is sandy to sandy-loamed textured, upon fractured bedrock mainly composed of granite. The vegetation is dominated by *Quercus rotundifolia* (holm oak), with other woody species such as *Lavandula stoechas* ssp. *pedunculata*, *Retama sphaerocarpa* and *Rubus* spp. Herbaceous vegetation can be divided in xerophytic pastures (on ridges, slopes and generally dry areas), and mesic pastures (on lowlands and water accumulation areas), with marked differences in productivity (low and high respectively). The management of the land is based on traditional practices typical for this agro-sylvo-pastoral-system, with periodic tree pruning to increase acorn and pasture production, rotational ploughing for cultivation and scrub encroachment control, as well as grazing by a transhumant herd of about 600 sheep. The geomorphology limits the application of these practices and promotes spatial heterogeneity in the ecosystem. The area is also specifically managed for small-game hunting (mainly rabbits), which includes cereal cultivation in some areas to be grazed by both rabbits and sheep.

Sampling Design

Study of Warren Location in the Landscape

A field survey was conducted between February and March 2002, and all warrens were identified and geo-referenced. A warren was defined as a group of burrows 2 m or less from each other. Vegetation and geomorphology layers were obtained from aerial orthophotos (1:5000) and ground confirmation. Given the scale of maps, all areas not covered by woody vegetation, rocks, paths or quarries were covered by herbaceous vegetation (patches of bare soil were not large enough to be included in the maps). Ploughing maps were obtained from aerial photographs (1:18000, 1:30000) covering the last 20 years. All maps were processed with ArcView GIS 3.2. A grid of 40 × 40 m cells was superimposed on all data layers, and sampling was carried out by randomly selecting 50% of the whole study area (550 cells, Fig. 1). From each cell the following variables were extracted: number of warrens, vegetation cover (in m^2) - holm oak (*Q. rotundifolia*), shrubs (*R. sphaerocarpa*), scrubs (*Lavandula stoechas* ssp. *pedunculata*), brambles (*Rubus* spp.), total herbaceous layer, and mesic pastures; cover of ploughed areas in the last 20 years (in m^2) according to ploughing frequency (never, 1–2, 3–4, >5 times), and time since the last ploughing event (2–7 years, 8–12, 13 or more years); cover of geomorphological classes (in m^2) - wet lowlands, sporadic water accumulation areas, runoff channels, low flat areas, high flat areas, slopes and ridges; cover of flood-prone areas (in m^2) rocks, stone heaps and stony ground. From the vegetation data, the following variables were derived: vegetation diversity (Shannon index, H') and evenness (E = H'/ln (richness)); and habitat heterogeneity index, E(P) = H'/ln (total no. of vegetation patches) (Rescia et al. 1994).

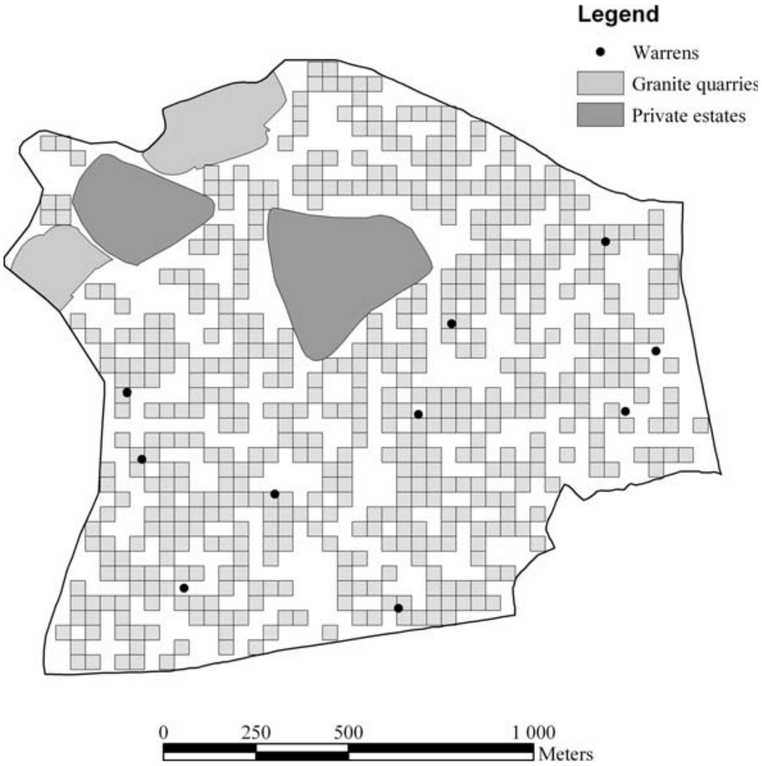


Fig. 1 Map of study area showing the sampled plots (40 × 40 m) for the study of factors affecting warren spatial distribution, and the warrens selected for rabbit effects on the vegetation community (*black dots*). The map includes two active granite quarries with no warren presence and two small private estates for which data on warren spatial location were not available

Study of Plant Community Changes Due to Warrens and Latrines

We sampled warrens and latrines to study plant community changes induced by rabbit activities inside their home ranges. For this purpose, a rabbit home range was considered as an area of imprecise outer limits surrounding a warren, and containing the highest proportion of latrines. Ten of these home range units were selected for vegetation surveys (Fig. 1), ensuring that the warrens were large (50–184 entrances). Large warrens were chosen because rabbit numbers correlate strongly with number of entrances (Cowan 1987; Palomares 2001), and a relatively high number of resident rabbits was desirable in order to observe their effects more easily. The vegetation was surveyed along four transects per warren, two following the line of maximum slope (upwards and downwards), and the other two approximately perpendicular to them. These were laid from the warren edge, outwards. Six 25 × 25 cm quadrats were laid at each transect at increasing distances from the warren:

0; 50 cm; 1.50 m; 3.50 m; 7.50 m and 15.50 m (see Fig. 2). The total number of rabbit pellets on each quadrat was used as an overall indicator of rabbit activity throughout the year (Wood 1988; Palomares 2001). This decision was based on a pellet degradation study performed in equivalent locations of the study area, which revealed that rabbit pellets could persist from a year up to 20 months (M. Rueda, unpublished data).

We investigated a total of 48 active latrines in the vicinity of eight of the warrens surveyed, at different distances from the warren (1.5 to 36 m; mean: 15.05 m) (Fig. 2). Vegetation data were collected on 10 × 10 cm quadrats, one in the central area of pellet accumulation, one in the surrounding vegetation ring (17–30 cm wide; mean: 23.29 cm) and a third one just beyond the vegetation ring. A final, "outside" quadrat was placed at approximately 1 m away from the external vegetation ring, and was considered out of the latrine's influence. Given that latrines were situated around the warrens that we had sampled, but at different distances from the warren centre, their "outside" position (see Fig. 2, latrine position no. 4) was surveyed using a 25 × 25 cm quadrat too, so that these data could be compared to the warren data. All latrine quadrats were at least 2 m away from any other latrine (range: 2–63.9 m, mean: 19.53 m) and from any of the warren transect quadrats.

In both series of quadrats, individual plant species were identified to evaluate floristic composition, and total percentage vegetation cover and mean vegetation height were measured as indicators of herbaceous community standing crop.

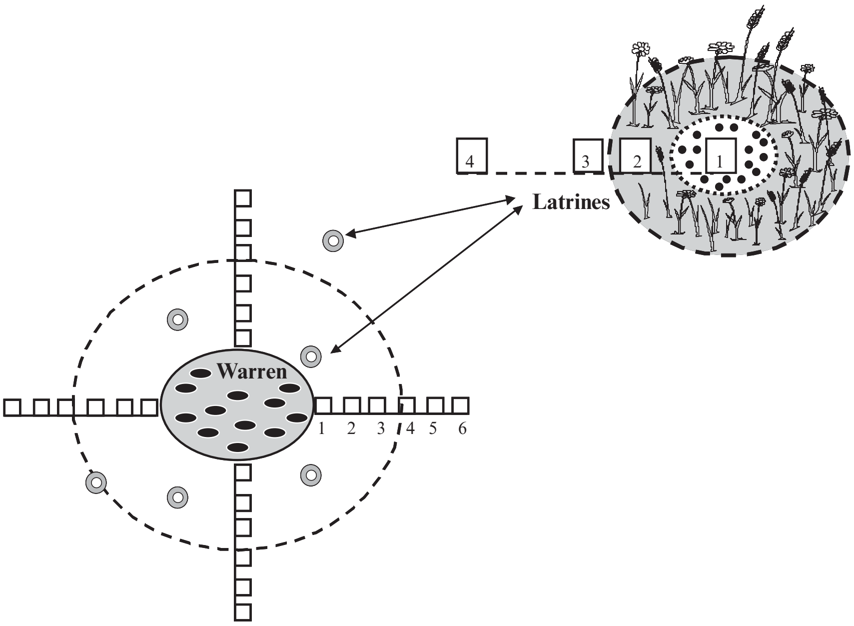


Fig. 2 Schematic sampling scheme for individual warrens and latrines (*not to scale*)

Data Analyses

Study of Warren Location in the Landscape

Multiple regression (forward stepwise) analyses were used to determine the main environmental factors affecting the location of rabbit warrens in the landscape. Warren density was the dependent variable, and the other 27 were the potential factors to be entered into the regression model. Due to the uncertainty in the statistical tests caused by the spatial autocorrelation among data (i.e., the non independence of data, and thus overestimated degrees of freedom), the response of warren density to each variable introduced in the final regression model was further tested by partial Mantel tests (Smouse et al. 1986). These are non-parametric analyses testing the relationship of two sets of variables (warren density and each predictor variable) while keeping constant the effect of a third (geographic position of plots). In this way, they are analogous to partial correlation, although the correlation coefficient calculated tends to be smaller due to the use of distance matrices instead of raw data. The significance levels are obtained via randomization procedures (see Oden and Sokal 1992; Legendre 2000 for details).

Study of Plant Community Changes Due to Warrens and Latrines

Differences in rabbit use (pellet counts) between warren transect positions were assessed using ANOVA. The herbaceous community was characterized by total vegetation cover, floristic composition, and mean vegetation height. "Floristic composition" was a synthetic variable obtained by applying a non-metric multidimensional scaling (NMS) ordination to individual species data. This is an iterative ordination technique that minimizes the stress of the k-dimensional configuration, taking into account the relationship between the dissimilarity (distance) in the original p-dimensional space and distance in the reduced k-dimensional ordination space (Clarke 1993). The distance matrix was calculated using the Sorensen index, which is more sensitive in heterogeneous data sets such as the one considered and giving less weight to outliers than Euclidean distances (Roberts 1986). For warren NMS analysis, species data from the "outside" position of latrines was added to transect data for comparison purposes. Differences in the herbaceous communities of both warrens and latrines were assessed using multivariate analysis of variance (MANOVA) performed in each case upon the three community parameters. As each home range was considered a unit, mean values per warren were used in the analyses, and warren identity was included as a block factor. In all cases, normality of data and homogeneity of variances were tested and data transformed where necessary. Statistical analyses were performed using PASSAGE 1.1 (M.S. Rosenberg 1998–2004), PCORD 4.25 (MjM Software Design 1995–1999) and SPSS 12.0 (SPSS Inc., 1989–2003).

Results

The regression model obtained for rabbit warren locations was highly significant ($R^2 = 0.2422$ $p < 0.0001$). Table 1 shows the variables included in the final model. Warren density responded positively to holm oak, bramble and rock cover, and to vegetation heterogeneity. It responded negatively to cover of flood-prone areas, relatively elevated topographic positions, high ploughing frequency, and long intervals between ploughing episodes. In spite of the spatial autocorrelation present among the data, the partial Mantel tests confirmed the significant effect of all variables on warren density except in the case of the geomorphological ones and long ploughing intervals (Table 1).

Pellet counts confirmed a significant gradient of increasing rabbit use towards the warren ($F_{5,45} = 4.555$; $p = 0.002$; Table 2, Fig. 3a). As expected, given the different location and size of surveyed warrens, block effect (warren identity) was significant for both warren transects ($F_{36,\ 159} = 3.972$, $p < 0.001$) and latrines ($F_{21,\ 55} = 2.086$, $p = <0.0001$). Once this effect was taken into account, the MANOVA results showed highly significant differences in

Table 1 Results for the multiple regression (forward stepwise) analysis on factors affecting warren density, and partial Mantel tests for the variables entered into the model (significance levels were obtained from a series of 1,000 randomizations)

	Regression analysis					Mantel test	
	Beta	Partial Cor.	Semipart Cor.	t(539)	*p*-level	r	*p*-level
Holm oak area	0.2632	0.2033	0.1791	4.820	<0.001	0.2495	<0.001
Habitat heterogeneity index	0.1213	0.1016	0.0881	2.372	0.0180	0.1049	<0.001
Bramble area	0.0899	0.1015	0.0880	2.369	0.0182	0.1054	0.015
Rock area	0.1052	0.0953	0.0826	2.223	0.0266	0.1663	<0.001
Ploughed ≥13 years ago	−0.1093	−0.1206	−0.1048	−2.822	0.0049	−0.0437	0.1060
Sporadic water accumulation area	−0.0848	−0.0970	−0.0840	−2.262	0.0241	−0.0345	0.2550
Ridge area	−0.0944	−0.1035	−0.0897	−2.415	0.0161	−0.0198	0.5090
Flood-prone area	−0.1039	−0.1083	−0.0940	−2.529	0.0117	−0.0564	0.042
High flat area	−0.0845	−0.0942	−0.0816	−2.196	0.0285	−0.0359	0.2350
High ploughing frequency	−0.0806	−0.0900	−0.0779	−2.097	0.0364	−0.0622	0.045

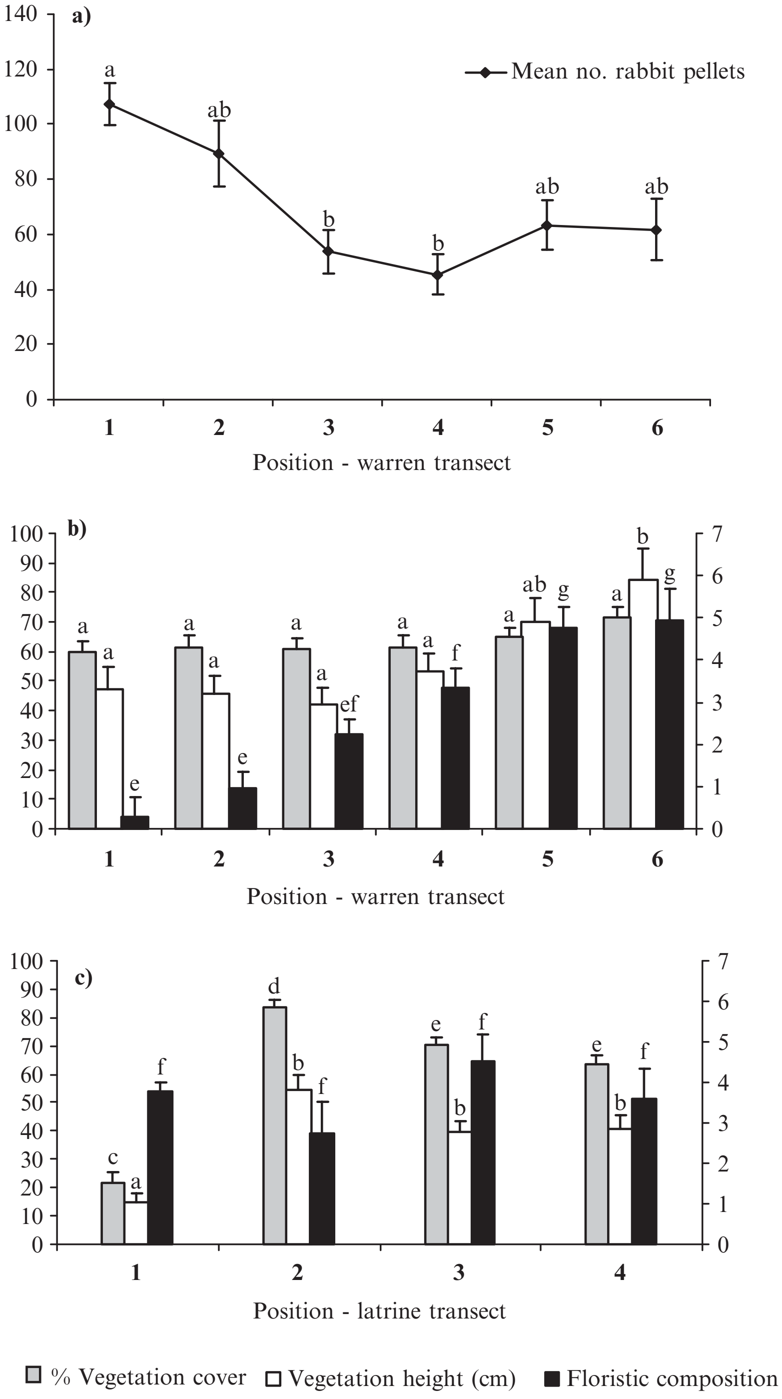
Table 2 ANOVA results for differences in community parameters between positions for warrens and latrines. The synthetic variable "floristic composition" corresponds to the first axis of NMS ordination. (* = $p<0.05$; ** = $p<0.01$; *** = $p < 0.001$; *NS* non-significant result)

Parameter	Warrens	Latrines
	F	*F*
Floristic composition	31.321***	1.005 NS
Mean vegetation height	7.268***	15.002***
Vegetation cover	1.253 NS	79.196***
Number of rabbit faecal pellets	4.555**	-

plant community parameters between transect positions in both cases (Wilks'Lambda, $F_{20,\ 140} = 6.869$, $p < 0.001$ and $F_{9,\ 46} = 10.315$, $p < 0.001$, respectively). This is depicted by axis I of the NMS ordination (Fig. 4), in which the arrow shows how floristic composition follows a parallel trend to rabbit use. This meant the replacement of several species (e.g., *Stellaria media* and *Urtica urens*), and most of all a change in the relative abundance of many others (e.g., *Spergularia rubra, Vulpia* spp. and *Hypochoeris glabra*). There were also significant differences in vegetation height, which followed the opposite trend: the vegetation was taller further away from the warren. Changes in herbaceous cover also followed this trend, but this result was not significant (Fig. 3b).

The central position of the latrines was an area of pellet accumulation with hardly any vegetation (only a few species had a mean cover greater than 1% in this area). Vegetation height was never above 4 cm due to constant mechanical disturbance by rabbits. This was in contrast to the surrounding vegetation ring where the herbaceous community was both significantly taller and denser compared to both the inside and outside positions of the latrine. Changes in floristic composition show a slight non-significant trend (Fig. 3c), due to a change in relative abundance of many species along the transect (e.g., *S. rubra*, *H. glabra*), with the replacement of some others (e.g., *Erodium cicutarium*, *Cerastium glutinosum*).

Fig. 3 Effects of distance from warrens and latrines on various parameters (floristic composition data have been transformed ((NMS coordinate + 50) × 100) to enable graphical presentation). **a)** Mean (±SE) number of counted pellets at various distances from warrens (1 = near ; 6 = furthest away). **b)** Mean (±SE) vegetation height (cm), % vegetation cover and floristic composition at various distances from warrens (1 = near ; 6 = furthest away). **c)** Mean (±SE) vegetation height (cm), % vegetation cover and floristic composition at various distances from latrines (1 = central area, 2 = vegetation ring, 3 = just outside vegetation ring, 4 = 1 m away from vegetation ring). Significant differences between transect positions were investigated using Tukey's post hoc test. *Bars* with equal letters are not significantly different


a)
Mean no. rabbit pellets
0
20
40
60
80
100
120
140
a
ab
b
b
ab
ab
1
2
3
4
5
6
Position - warren transect
b)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
a
a
e
a
a
e
a
a
ef
a
a
f
a
ab
g
a
b
g
1
2
3
4
5
6
Position - warren transect
c)
0
10
20
30
40
50
60
70
80
90
100
0
1
2
3
4
5
6
7
c
a
f
d
b
f
e
b
f
e
b
f
1
2
3
4
Position - latrine transect
▨ % Vegetation cover
☐ Vegetation height (cm)
■ Floristic composition

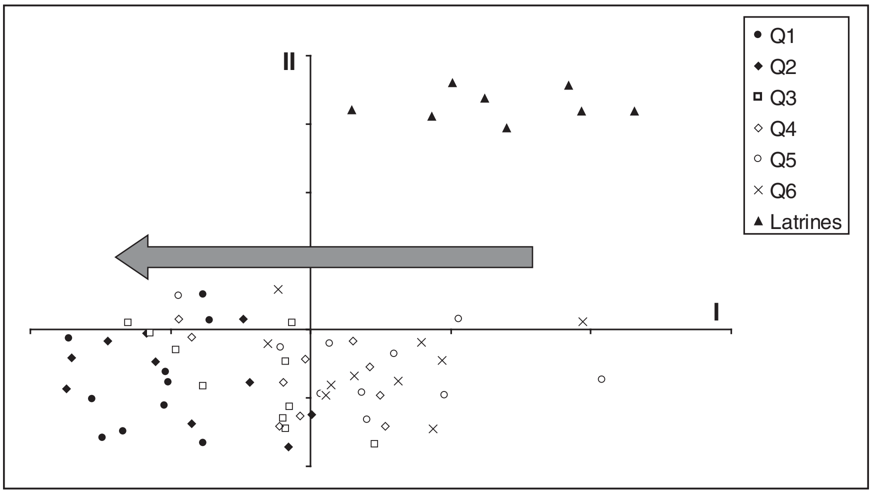


Fig. 4 Results of NMS performed upon species abundance data for each warren transect position and the outer position of latrines. ● Q1 = position 1; ◆ Q2 = position 2; □ Q3 = position 3; ◊ Q4 = position 4; ○ Q5 = position 5; × Q6 = position 6; ▲ Latrines = outer position of latrines. The *arrow* indicates the gradient captured by the first axis, which also reflects rabbit use measured as number of rabbit faecal pellets found in each quadrat

An additional result from NMS ordination for the warren influence area showed that floristic composition was very different between latrine and warren quadrats at any of the warren transect positions, despite the fact that the surveyed latrines were situated at different distances from the warren. Figure 4 shows further results of NMS ordination, where axis I represents differences in floristic composition between warren transect positions, and axis II represents differences between warren positions and the outside position of latrine transects, showing that floristic composition is still under the latrine's influence despite these quadrats being 1 m away. This effect is reinforced by the fact that latrine distance to the warren did not have an effect on herbaceous community parameters (floristic composition: r = –0.004, $p = 0.978$; vegetation height: r = –0.285, $p = 0.052$; vegetation cover: r = –0.090, $p = 0.648$).

Discussion

Results showed that certain habitats within the dehesa landscape were preferred or rejected by rabbits for warren building. Wet lowland areas seem to be avoided by rabbits for warren construction, as they are flood-prone due to their topographic position and excess clay. Parer and Libke (1985) reported that flooding is negative for the survival of pups and young rabbits, although it does not seem to affect adult individuals. This selective mortality and the associated flooding and collapsing of warrens would have important consequences for local rabbit populations (Palomares 2003a).

Most warrens were concentrated in areas of high woody vegetation cover and habitat heterogeneity. Many authors have already highlighted the importance of both trees and shrubs as providers of refuge for rabbits to reduce predation (Moreno et al. 1996; Lombardi et al. 2003; Palomares 2003b), and also as a possible structural support for their burrow systems in areas of unstable soils (Palomares 2003b). Woody species (especially trees and shrubs) in arid and semi-arid environments promote nutrient enrichment and amelioration of microenvironmental conditions under their canopies (Archer et al. 1988; Vetaas 1992; Callaway 1995; Callaway and Pugnaire 1999). However, this potentially higher productivity can be counteracted by an excess of shade when the canopy is too dense, which reduces herb density. For rabbits, both cover and food are important. Hence, it would be necessary to have separated complementary zones, i.e., a closed area for refuge and an open area for grazing, or else to have them interspersed. The literature provides examples of both cases for the Iberian Peninsula (Moreno and Villafuerte 1995; Lombardi et al. 2003). In the current study, rabbits preferred the most interspersed situation: the positive response of warren density to both holm oak cover and the heterogeneity index implies that rabbits prefer the most fragmented parts of the landscape.

Traditional management practices are essential for the maintenance of dehesa structure. Among them, ploughing is used to prevent shrub encroachment, and generally involves cereal cultivation both as the initial step in the pasture generation process and as fodder for herbivores (Spiers 1981; Joffre et al. 1988). In those dehesa ecosystems with the additional aim of game exploitation, rabbit grazing in these crop fields is explicitly considered. In our study site, besides mowing of productive zones and tree pruning, ploughing is an important factor regarding warren location. Excessively high ploughing frequencies negatively affect warren location, probably because of the changes induced in the herbaceous community by frequent disturbance, and the peril of warren destruction in the process.

Environmental factors operating at landscape or ecosystem scale regarding refuge and pasture availability depend on both geomorphological and management characteristics. These factors condition warren location, whereas rabbit engineering activities have effects at a lower scale. Within each warren home range, rabbits introduce further heterogeneity in the ecosystem through their different activities, affecting mainly, but not only, the herbaceous community. Results showed significantly greater rabbit activity in the vicinity of warrens than further away. This resulted in lower vegetation height due to constant trampling and herbivory, but no differences in vegetation cover between positions near the warren and those further away. Floristic composition varied with distance from warrens, which implies that some species disappeared or decreased in abundance near warrens, probably because they succumbed to rabbit herbivory and disturbance. Other species were favored by the warren's influence. Changes in floristic composition were coupled with differences in plant growth forms. Near the warren dwarf, prostrate, creeping and rosette growth forms were observed, which explains why vegetation height is lower and how cover is maintained despite intense rabbit activity in this area. This is consistent with Gillham's (1955) records of a higher proportion of such plant growth forms found in the vicinity of rabbit warrens in the Pembrokeshire islands.

The herbaceous community associated with latrines followed a slightly different pattern. Mechanical disturbance seems to be the main factor operating in latrines, since the main significant differences are observed in the central area, which is subjected to trampling and scratching by rabbits. Extra nitrogen from feces and urine may be responsible for an increase in vegetation cover and height in the surrounding vegetation ring (Floate 1981), but do not appear to have dramatic effects on floristic composition at a first glance, given the nitrophilous nature of some of the recorded species. However, ordination results for floristic composition suggest that leached nutrients could exert their influence beyond the distance (1 m) considered in this study, since the area around latrines is clearly different in floristic composition from the other surveyed positions within the rabbit's home range. This suggests that latrines may be providing new opportunities for plant germination and establishment. In fact, other studies about the effects of latrines on Mediterranean soil properties have concluded that they are an important locus of organic matter and enhance soil chemical fertility and plant growth in semi-arid Mediterranean ecosystems, thus promoting diversity and a greater stability of the system (Willot et al. 2000; Petterson 2001).

Conclusions

This chapter demonstrates that warrens and latrines support a distinct annual plant community. The general processes involved in the shaping of these communities, soil disturbance and pellet accumulation, are common to all mammalian herbivores, and have a deep impact on the vegetation (Harper 1977), irrespective of local plant species assembly and biogeographical zone. Although some of those effects can be species-specific, the final result is a change in some, if not all, the community parameters analyzed in this study (floristic composition, total vegetation cover, and mean vegetation height). These engineering effects act as bottom-up forces that may influence community structure and the behavior of other species (Bangert and Slobodchikoff 2000). This role is twofold: for herbaceous species, which take advantage of the spaces created by rabbit disturbance and herbivory or nutrient enrichment; and potentially for other species that can use these different plant assemblages and strata as resources themselves (e.g., insects). Latrines have already been typified as an important niche for endemic dung beetles (Verdu and Galante 2004) and warrens can be used as a temporary refuge or even be taken over by other species, for example reptiles (Blazquez and Villafuerte 1990) and other mammals (Villafuerte, pers. comm.). In several places around the world, European rabbit plasticity and colonizing ability has lead them to reach pest status. However, in the resource-limited ecosystems of the Mediterranean basin, rabbits are prey for more than 20 predators, and in some cases they comprise more than 40% of the consumed biomass (Delibes and Hiraldo 1981). Therefore, rabbits are a keystone species and, as our results show, have the potential to increase biodiversity at different scales.

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Forest-Fire Regime: The Missing Link to Understand Snowshoe Hare Population Fluctuations?

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Introduction

The snowshoe hare (*Lepus americanus*) is actually recognized as a keystone species in North American boreal forests, having significant effects on vegetation and being one of the most relevant preys for several predators (Keith 1963; Keith and Cary 1991; O'Donoghue et al. 1998; Krebs 1996; Krebs et al. 2001a). According to the bottom-up theory, prey species at the base of the food chain are critical to maintain wildlife biodiversity at the landscape level (Noss 1987; Hunter 1999; Thompson 1988; Thompson and Angelstam 1999).

Population fluctuations in the snowshoe hare have thus fascinated biologists over the last 50 years (see Keith 1963 and 1990; Lidiker et al. 2000; Murray 2000; Krebs et al. 2001b). The snowshoe hare exhibits an 8- to 11-year cycle in the northern part of its geographical range, but this cycle is dampened or eliminated in the southern part of its range (Keith 1990; Murray 2000). Another characteristic of these fluctuations is that population trends are largely synchronous over large geographical regions (Murray 2000; Krebs et al. 2001b).

Numerous studies have explored this cycle and its causes, linking the hare cycle to exogenous or endogenous causes (see review in Murray 2003). Hypotheses in the first group (Murray 2003) are solar activity affecting plant productivity (Sinclair et al. 1993; Ranta et al. 1997; Sinclair and Gosline 1997), weather changing habitat quality (Grange 1949; Butler 1953), ultraviolet rays and forest fires modifying plant succession and composition (Rowan 1950; Grange 1965; Fox 1978), and plant nutrients varying naturally and cyclically (Lauckhart 1957). All these hypotheses emphasize that external causes affect food availability or quality for the snowshoe hare and consequently its population densities; the effect is bottom-up. Possible endogenous causes are numerous (Murray 2003): epidemics due to infectious diseases or shock (MacLulich 1937; Green and Larson 1938), physiological stress at high density

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reducing fitness (Christian 1950), polymorphic behavior according to population density (Chitty 1967; Krebs et al. 1992), overbrowsing at high density (Lack 1954), plant defenses induced by browsing (Fox and Bryant 1984; Bryant et al. 1985), predation according to density (Trostel et al. 1987; Royama 1992), and finally interaction between food shortage and predation (Keith 1974, Krebs et al. 1995; Sinclair et al. 2000). All hypotheses from this second group are based on intrinsic causes; some refer to predation whereas some other are based on food or an interaction between the two. Bottom-up as well as top-down hypotheses could thus explain the processes underlying this phenomenon (Keith 1963, 1974, 1990; Keith et al. 1984; Krebs et al. 1995, 2001a). The relative importance of these causes may vary in time, and the hare cycle is probably the resultant of the interaction among several of them.

Among all these causes, the most accepted point of view is the one taking into account food and predation. Keith (1974) have proposed that successive hare–food and hare–predator interactions cause declines of cycling hare populations, an hypothesis also tested and verified by Wolff (1980) in Alaska. In this experiment, Wolff (1980) have suggested that a steep increase in hare abundance can lead to overbrowsing and subsequently to individual shifts from optimal to suboptimal habitats where predation risk is higher. Reviewing causes of the snowshoe hare cycle, Keith et al. (1984) have concluded that starvation (bottom-up effect) can be considered as the cause of the first phase of population decline, followed by an increasing effect of predation, leading to a relative equilibrium between food and predator influence, and finally by a last period during which predation is solely responsible of the decline in abundance. Krebs et al. (2001a) have later considered that the three trophic levels are clearly involved in the control of those fluctuations: vegetation (hare food supply), snowshoe hare (as herbivore and prey) and predators. They have suggested that the 10-year cycle of the snowshoe hare is the result of the interaction between predation and food supply, but of these two factors, predation is clearly the dominant process. By comparing two hare populations found on an island and on close mainland sites, Krebs et al. (2002) have demonstrated the importance of predation as a major cause of hare population trends. Indeed, island-confined population exhibited lower amplitude of abundance fluctuations, and higher juvenile and adult survival rates during most years, except during years of population declines. Moreover, they observed that the predator guild exhibited lower densities and species richness on the island than on the mainland sites.

In a 16-year breeding experiment where they produce purebred lineages of high-phase and low-phase females, Sinclair et al. (2003) have recently observed that high-phase females exhibited lower reproductive rate, lower reproductive output, and earlier senescence than low-phase females. They thus concluded that an intrinsic mechanism operates in synergy with extrinsic causes of the 10-year hare cycle (predation and food shortage) in cycling populations.

The lower numerical variance in southern hare populations, which dampens the hare cycle, is considered to be the consequence of higher and steadier predation rates by a group of generalist predators typically common at lower latitudes (Howell 1923; Wolff 1980, 1981; Buehler and Keith 1982; Keith and Bloomer 1993; Stenseth et al. 1997). Several studies also suggest that the interaction between predation and food can explain the absence of snowshoe hare cycle in the south-eastern part of its distribution range, where richer forest ecosystems support constant predation pressure from generalist and specialist predators, along with constant and high food productivity (Keith 1990; Wirsing et al. 2002).

Krebs et al. (2001b) have suggested two general models to explain synchrony of hare population fluctuations across large geographic regions: one driven by weather and one by dispersal movements. Sinclair et al. (1993) have proposed a weather model, related to the sunspot cycle, to explain synchronicity of the hare cycle across North America. Sunspots and hare numbers are indeed highly correlated for three time periods during the past 250 years: 1751–1787, 1838–1870, and 1948–1986, which were all periods of high-amplitude sunspot fluctuations (Sinclair and Gosline 1997). Krebs et al. (2001b) have suggested that because sunspots affect broad weather patterns, they might, through weather, entrain snowshoe hare cycles across the continent when solar activity is unusually high. However, Sinclair et al. (1993) and Krebs et al. (2001b) were unable to put forward a mechanism for sunspot-affected weather patterns to translate into demographic impacts on hares.

Another interesting facet of hare cycle studies concern the temporal association between hare habitat and hare population level. The relationship between understory cover (as a protecting visual barrier against predators) and snowshoe hare abundance has been studied extensively and has clearly shown that the intensity of habitat use is positively correlated to lateral cover density (Grange 1932; Adams 1959; Bider 1961; Conroy et al. 1979; Wolff 1980; Orr and Dodds 1982; Wolfe et al. 1982; Pietz and Tester 1983; Parker 1984; Carreker 1985; Monthey 1986; MacCraken et al. 1988; Koehler 1991; Ferron and Ouellet 1992; Ferron et al. 1998; Litvaitis 2001; Beaudoin et al. 2004). Such studies have concluded that hares prefer feeding and hiding cover presenting a dense (>80% obstruction at 15 m) lateral cover, and use travel cover under dense canopy. Predation rates on hares are lower in dense cover than in sparser understory (Wolff 1980).

Surprisingly, the link between habitat selection and demography in the snowshoe hare has not yet been investigated. It is actually known that local and regional fluctuations in population abundance can be related to movements of hares searching for a habitat of better quality. Indeed, at high hare density, subordinate animals are likely driven into more open habitat were they are more vulnerable to predation, according to a small scale adaptation of multi-nodal diffusion model (Smith 1983) and individual-oriented model (Bascompte et al. 1997). At the landscape level, the availability of good cover is greatly dependent on the presence of regenerating forest stands. In the boreal forest, forest fires (and more recently logging) create those successional stages ideal for snowshoe hares (Radvanyi 1987). As fire has been a dominant disturbance regime in the Canadian boreal forest since the last ice age (Stocks et al. 2003), it can be presumed that the snowshoe hare has become adapted to it by being demographically opportunistic. Our objective is thus to establish correlative evidence of a relationship between geographical location, sunspot incidence, forest fire regime, and variations in hare abundance across Canada.

Methodology

We gathered data on hare abundance time series using different indices (i.e., bag count, hunting success, pellet count, number of furs sold); we used published data or graphs to estimate the amplitude of fluctuations in abundance or used databases from colleagues or from our research team. We considered only time series covering at least one period of abundance fluctuation (i.e., 10 years or more), the longest one covering 12 periods. For each time series, we first log-transformed hare abundances to minimize bias related to harvest statistics. Mean amplitude of fluctuations in abundance was calculated for a given time series by averaging cycle amplitude between successive minima and maxima.

Forest fire regime was calculated using the large fire database (LFDB) used by the Canadian Forest Service, which includes information such as annual fire abundance, fire location, and burned area for all fires larger than 200 ha in area censused in Canada for the 1959–1997 period, whatever they were caused by lightening or human activities. The LFDB divides Canada into territorial units according to three different spatial scales: ecozones ($n = 15$; mean area = 838,516 km^2), ecoregions ($n = 219$; mean area = 57,962 km^2) and ecodistricts ($n = 1{,}031$; mean area = 12,223 km^2). As the origin of hare time series were not always clearly identified, we used the ecoregion spatial scale as an interesting compromise between low geographical precision and high precision in fire regime calculation. We were thus able to define relative fire regime ranging from 1,000 to 1.6 million years, for each of these ecoregions (Fig. 1).

The geographical location of each time series of hare abundance was established with a GIS using the same ecoregions as for the LFDB, and a corresponding fire regime was thus paired to each time series. Pearson or Spearman ranked-correlations were used to test relationships between mean amplitude value in hare abundance fluctuation, latitude, longitude and fire regime, and between number of fires, total burned area and sunspot abundance (Legendre and Legendre 1998; Zar 1999). Number of fires, total burned area, and number of sunspots were previously standardized by the mean to be

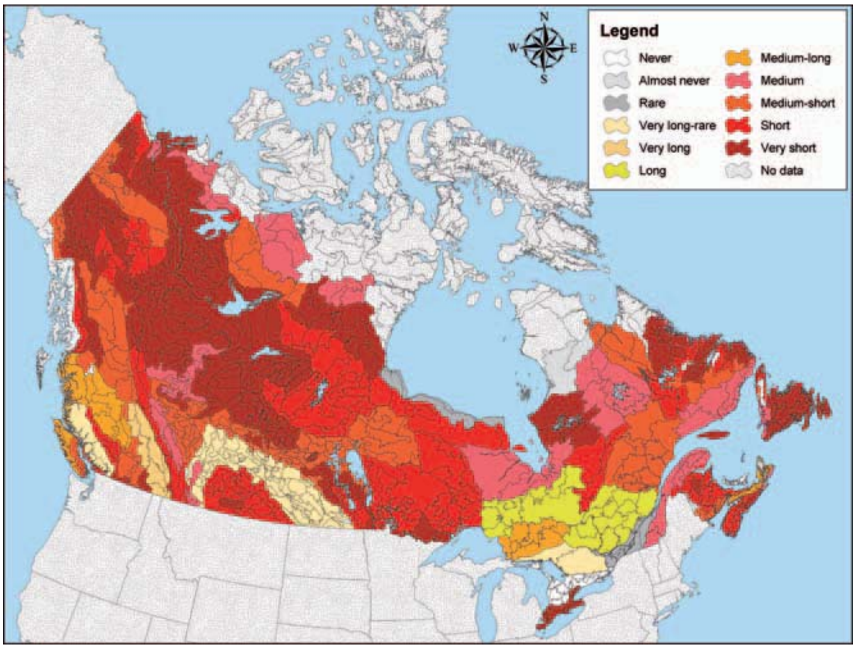

Fig. 1 Distribution of forest fire regime across Canada (large fire database (LFDB) from the Canadian Forest Service: all fires >200 ha during the 1959–1997 period)

compared on a similar scale. Data on annual sunspot abundance between 1959 and 1997 were freely downloaded from the Royal Observatory of Belgium website (http://sidc.oma.be/), which provides the Sunspot Index Data Center.

Results

We found no correlation between annual sunspot abundance and number of fires ($r_p = 0.24$; Bartlett $\chi^2 = 2.33$; $p = 0.13$; $P = 0.33$), nor between sunspot abundance and total burned area between 1959 and 1997 ($r_p = 0.22$; Bartlett $\chi^2 = 1.92$; $p = 0.17$; $P = 0.28$) (Fig. 2). However, total burned area and sunspot abundance were related before 1990 ($r_p = 0.55$; Bartlett $\chi^2 = 10.08$; $p < 0.01$; $P = 0.90$).

The amplitude of fluctuations in hare abundance in the boreal forest was correlated to latitude ($r_p = 0.61$; Bartlett $\chi^2 = 6.66$; $p = 0.01$; $P = 0.78$), but not to longitude ($r_p = –0.39$; Bartlett $\chi^2 = 2.42$; $p = 0.120$; $P = 0.36$) (Fig. 3). Forest fire regime was influenced by latitude (Zk $r_p = –0.68$; Bartlett $\chi^2 = 8.91$; $p < 0.01$; $P = 0.89$) but not by longitude ($r_p = 0.34$; Bartlett $\chi^2 = 1.73$; $p = 0.189$;

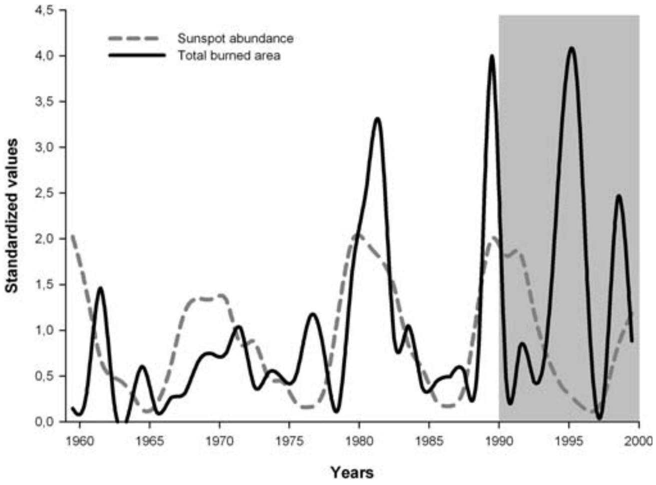

Fig. 2 Relationship between annual sunspot abundance and total burned area, between 1959 and 1999. *Shaded area* refers to years following 1990, for which correlation between the two variables decreased

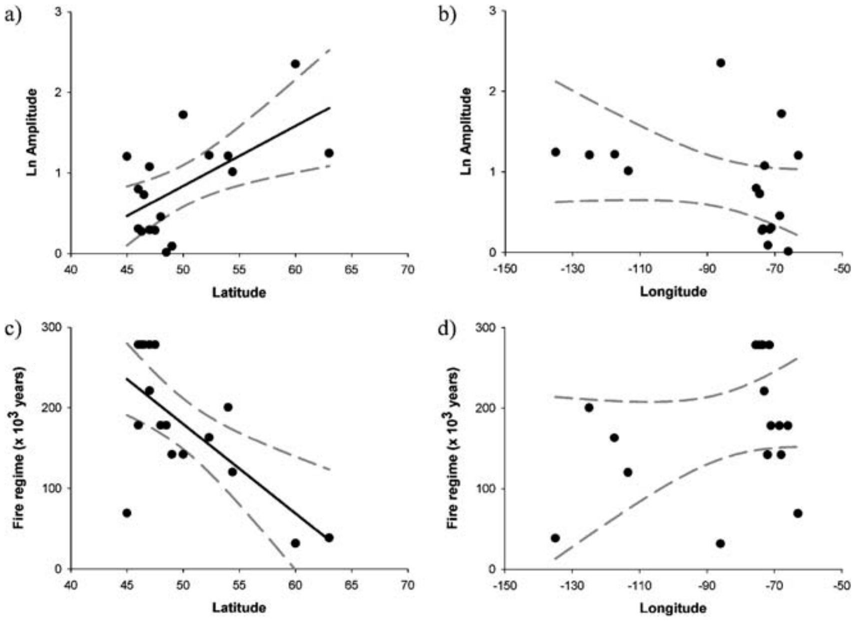

Fig. 3 Relationships between mean amplitude of hare fluctuations and latitude (a) or longitude (b), and between ecoregion fire regime and latitude (c) or longitude (d). Solid black lines represent significant relationships while dashed grey lines refer to 95% confidence intervals

P = 0.27) (Fig. 3). Finally, the mean amplitude of fluctuation in hare abundance was negatively correlated to the length of forest fire regime in the Canadian boreal forest ($r_p = -0.60$; Bartlett $\chi^2 = 6.99$; $p < 0.01$; $P = 0.77$)

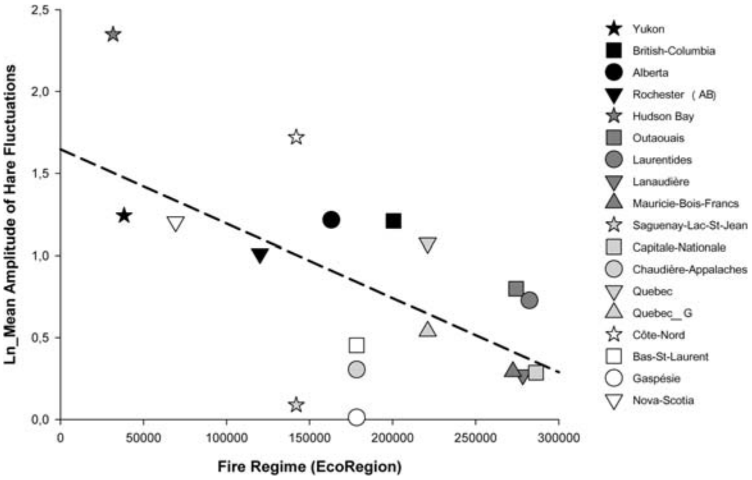

Fig. 4 Mean amplitude of hare abundance fluctuations in relation to forest fire regimes. Each *point* corresponds to a different hare time series, and *colors* refer to a longitudinal gradient from west (*black*) to east (*open white*). Sources of hare time series data: Yukon, British Columbia, Rochester and Alberta (Krebs et al. 2001b); Hudson Bay: (MacLulich 1957); Bas-St-Laurent, Saguenay-Lac-Saint-Jean, Capitale-Nationale, Mauricie-Bois-Francs, Outaouais, Côte-Nord, Gaspésie, Chaudière-Appalaches, Lanaudière, Laurentides and Quebec_G: Godbout (1999); Nova-Scotia and Quebec: Murray, D.L. Data gratefully provided

(Fig. 4). This suggests that forest fires, through their effects on hare habitat, directly influence hare abundance.

Discussion

Analyses of available data on hare populations, forest fires, and sunspots suggest that there are correlative evidences of relationship between these variables across the North American boreal forest.

Sunspot abundance is cyclic and presents superimposed periodicities of 11 and 22 years (Sinclair et al. 1993; Klvana et al. 2004). The present study indicates that at the continental scale, the total burned area in the boreal forest between 1959 and 1990 was related to the annual abundance of sunspots, but this relationship did not hold after 1990 (Fig. 2). There is growing evidence that a link exists between sunspot abundance and weather but the exact mechanism underlying this relation is still unclear (Rind 2002). The incidence of forest fires, which is the main stand-replacing process in the boreal forest, is greatly influenced by weather (Stocks et al. 2003). Fires thus generate more or less abundant regenerating stands depending on weather. Regenerating stands presenting a dense lateral cover are known to be a prime habitat for the snowshoe hare as they provide food and protection from predators (Brocke 1975; Conroy et al. 1979; Wolff 1980; Parker 1984; Monthey 1986; Ferron and Ouellet 1992; Ferron et al. 1998; Litvaitis 2001). Consequently, there is possible link between sunspot abundance and snowshoe hare habitat through a sunspot–weather–forest fires–hare habitat relationship. This relationship is all the more interesting as periodicities of sunspots and hares are similar. Indeed, Sinclair et al. (1993) have previously pointed out that hare numbers are correlated with sunspot numbers and that there is a 10 to 11-year periodicity in the correlograms. They also suggested that the snowshoe hare cycle is modulated indirectly by solar activity through an amplified climate cycle that affects the whole boreal forest. However, they, as well as Krebs et al. (2001b), were unable to put forward a mechanism for sunspot-affected weather patterns to translate into demographic impacts on hares. The relationship suggested here might be the missing link in the understanding of this large-scale zeitgeber of snowshoe hare population fluctuations. Some other indications suggesting that this may be the case can be found in the literature. Fox (1978) has pointed out a coincidence between the Canada lynx cycle, an important predator of the snowshoe hare, and the occurrence of forest and brush fires. Grange (1965) has suggested that fires set in motion plant succession, potentially leading to an increase in snowshoe hares. Another interesting facet of our hypothesis is that it also takes into account the synchronicity of hare cycles across large areas of North America; the effect of sunspots on weather, and ultimately on forest fire, is indeed a continental phenomenon (Krebs et al. 2001b).

Our results indicate that there is however a lack of coincidence between sunspot abundance and total burned area in the boreal forest after 1990. Similarly, Murray (2003) have reported that synchrony in hare population cycle across North America have recently declined. He also indicated that the cause of this decoupling is under investigation as the exact cause of this phenomenon is still unknown. Weber and Flannigan (1997) have recently suggested that climate change is changing fire regime in the boreal forest. This recent perturbation of the boreal ecosystem along with increasing logging activities may have contributed to desynchronize hare population cycle across the continent.

Fire intensity rapidly responds to local weather and regional climate (Weber and Flanagan 1997). The fire regime thus naturally differs across different zones of the boreal forest. The LFDB supports this conclusion. The present study suggests that the amplitude of the snowshoe hare cycle apparently reacts, at least at the regional scale, to the length of the fire regime (Fig. 4). We suggest that at the landscape level, if the fire regime is very long, the availability of good habitat, that is regenerating stands, is limited but relatively constant over time. However, in other boreal ecoregions where the forest fire cycle is very short, good habitat will be more available, providing more opportunity for exponential growth of hare populations. Consequently, hare density will be more constant in the former situation whereas it will be more variable in the latter case. Despite the fact that forest fires occur in other often more fertile forest ecosystems of Canada, they are not an essential stand-replacing process as in the boreal forest. Using paired data on hare abundance time series and fire regime in those ecosystems, we were thus not surprised to find no correlation under such conditions.

Our study also reveals that forest fire regime was influenced by latitude and that amplitude of fluctuations in hare abundance in the boreal forest was also correlated to latitude (Fig. 3). The north-south gradient of reduction in amplitude of hare fluctuations is in accordance with Murray's (2000). In the boreal forest, forest fires may contribute to this dampening, as suggested by our analyses. In more southern forest ecosystems, the most prevalent hypothesis is that lower population densities and increased stability are probably related to the effects of strong and consistent predation by a suite of facultative and generalist predators, combined with the greater patchiness of suitable hare habitat (Wolff 1980, 1981). Recent investigations have however failed to exhibit clear north–south disparity in survival and reproductive rates of hares, and it is consequently unclear which factor causes this demographic trend at the continental level (Murray 2003).

Regenerating stands are an excellent feeding habitat for the snowshoe hare (Pease et al. 1979; Litvaitis et al. 1985; MacCraken et al. 1988; Smith et al. 1988; Scott and Yahner 1989; De Bellefeuille et al. 2001), and their availability not only reduces predation risk but also provides abundant food, another condition favoring population growth. The relation between forest fire regime and the amplitude of hare population cycle in the boreal forest thus supports the conclusion of Krebs et al. (2001b) that fluctuations of snowshoe hare population are the result of the interaction between predation and food supply.

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Introduced Lagomorphs as a Threat to "Native" Lagomorphs: The Case of the Eastern Cottontail (*Sylvilagus floridanus*) in Northern Italy

Anna Vidus Rosin, Nicola Gilio, and Alberto Meriggi*

Introduction

Eastern cottontails were introduced in Pinerolo (southwest of Torino), Italy, near the Pellice River, in 1966 (Spagnesi 2002). By the 1980s, the population expanded into the region of Piemonte with localized groups in the province of Alessandria (Prigioni et al. 1992), reaching 25–27 cottontails/km^2 in the late 1990s, and the percentage of cottontails in the total of all mammals killed on roads rose from 2.2% in 1995 to 6.0% in 1997 (Silvano et al. 2000). At the end of the 1990s, the species was present in the western part of Lombardia and in some localities in the south side of the Po River (Fig. 1). Presently, Eastern cottontails are also present in the regions of Veneto, Emilia Romagna, Marche, and Toscana in response to population growth and additional releases to increase the potential for small game harvesting. Meriggi (2001) observed that in release sites, cottontails preferred the successional and weedy vegetation with dense brushy cover along rivers where they can find cover from predators. He also speculated that cottontails might compete with native Lagomorphs (European hare, *Lepus europaeus*, and European rabbit, *Oryctolagus cuniculus*), especially in poor habitats, limiting their population size to a low level. A prerequisite of competition would be that Eastern cottontails have similar habitat requirements as other native Lagomorphs. In this chapter, we analysed the potential competition between Eastern cottontails, European hares and European rabbits by investigating the habitat requirements of Eastern cottontails in two areas of northern Italy.

Materials and Methods

Study Areas

We studied cottontails in two areas (A: 45°05 N, 8°42 E and B: 45°15 N, 8°56 E) located between the Ticino and Po Rivers. The climate was similar in both areas; annual rainfall averaged 800 mm and was concentrated in spring and

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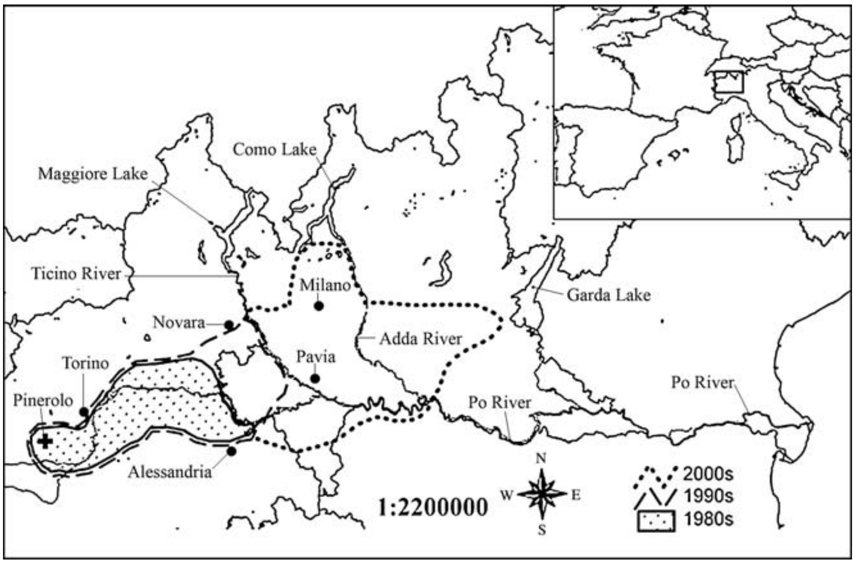

Fig. 1 Eastern cottontail expansion in northern Italy

autumn. The yearly temperature averaged 12°C (January 1.0° and July 22.5°C). The two study areas were characterized by similar plant communities and different cottontail densities as estimated by line-transect censuses (study area A: 65.6 cottontails/km^2, SD = 5.64, mean transect length of 15.9 km, $n = 30$; study area B: 35.6 cottontails/km^2, SD = 5.84, mean transect length of 21.2 km, $n = 20$) carried out during the first 2 h after sunrise and before sunset, in late spring–early summer, when we observed the greatest probability of detecting cottontails (for details on the method see Buckland et al. 2001). In both study areas, European rabbits were absent and hares were occasionally observed (study area A: 0.1 hares per km of transect; study area B: 0.03 hares per km). Study area A (325 ha) included croplands (71.4%), especially rice (50.4%), maize (9.1%), winter cereals (6.4%) and legumes (5.5%). Natural vegetation was present in small mesophyll broad-leaved woods (8.7%), along hedgerows, field edges, ditches, and dirt roads (row habitats, 14.7%), and in fallow fields (0.7%). Farmsteads were 4.5% of the area. In study area B (416 ha), croplands dominated with rice (21.7%), maize (17.9%), and winter cereals (5.8%) the most common. A small area was used for poplar plantations (3.5%) and hay fields (1.0%). Woodlots were 25.6% of the area, row habitats 17.1%, and fallow fields 5.7%. Farmsteads only covered 1.8% of the area.

In woods of both areas, common overstory species included oaks (*Quercus robur peduncolata*), white poplars (*Populus alba*), black poplars (*Populus nigra*), alders (*Alnus glutinosa*), willows (*Salix* spp.), hornbeams (*Carpinus betulus*), elms (*Ulmus campestris*), cherries (*Prunus avium*), and locusts (*Robinia pseudoacacia*). The understory plants included hazels (*Corylus avellana*), hawthorns (*Crataegus* spp.), brambles (*Rubus* spp.), elders (*Sambucus* spp.), and cornels (*Cornus mas*).

Methods

We mapped vegetative cover types in both study areas by direct surveys and then digitalized these with ArcView 3.2. We examined habitat use by cottontails from May 2002 to November 2003 using three different methods.

First, use was assessed by defining the distribution of cottontails in each study area. We selected 100 plots with a radius of 1 m that were randomly distributed. Within the plots, we determined the presence/absence of cottontails based on fecal pellets. Cottontail pellets were distinguished from European hare ones by their smaller size and from leveret ones by their different shape. Anyway, the hare density was so low in our study areas that the probability to find hare pellets in a given plot could be considered negligible. Plots were surveyed once a season. The range of cottontails was delineated by kernel analysis at 99 and 50% of occupied plots using program Ranges 6 V.1 (Kenward et al. 2003). We then compared the abundance of individual cover types in the range to their availability in the study area using Jacobs index of preference (Jacobs 1974):

$$PI_i = \left[(X_1/Y_1) - (X_2/Y_2)\right] / \left[(X_1/Y_1) + (X_2/Y_2)\right]$$

where X_1 is the surface of the i^{th} habitat type included in the range, Y_1 is the whole surface of the range, X_2 is the surface of the i^{th} habitat type included in the study area, and Y_2 is the whole surface of the study area. The index ranges from −1 to +1, taking positive values if the usage of the i^{th} cover type is greater than its availability and negative values if the usage is less than availability. To test the reliability of the Jacobs indices, we re-sampled the random points 1,000 times in each study area by the bootstrap method (Dixon 1993) and redefined the ranges by the kernel analysis at 99 and 50%. We then calculated the Jacobs index of preference for each bootstrap sample and for each habitat. Finally, we checked for the normality of indices distribution and tested for significant deviations of the index values from 0 (neutral selection) by the one-sample Student's *t*-test (Hesterberg et al. 2005).

Our second method included direct observations of cottontails within 100-m buffers along trails that we traveled weekly by car and designed to span both study areas. The observed frequencies and proportions of cottontails in each habitat type (observed usage proportions) were then calculated and compared with those expected (expected usage proportions) by means of χ^2 goodness-of-fit test and Bonferroni simultaneous confidence intervals analysis (Manly et al. 1993). We tested the null hypothesis that cottontails used cover types in accordance to their availability.

For our third approach, we compared the micro- and macro-habitat characteristics of the random plots with and without cottontail pellets using discriminant function analysis. We measured 11 micro-habitat variables within the 1-m radius plots and ten macro-habitat variables (percentage of habitat types) within a 100-m radius buffer from the plot centre (Appendix 1). Discriminant function analyses (DFA) were performed to differentiate plots with and without fecal pellets. Only those variables that differed between occupied and unoccupied sites (based on one-way ANOVA, $P < 0.10$) were entered in the DFA, as suggested by Green (1974) and Noon (1981). All cover-type analyses were conducted with ArcView 3.2. Statistical analyses were performed by SPSS/PC + Version 11.0.

Results

Fifty random plots out of 100 in study area A and 27 in study area B were occupied by cottontails. Considering the range delimited by the kernel analysis at 99%, woods and fallow fields were selected in both study areas, whereas legumes, farmsteads and row habitats were selected only in study area A, and maize fields and poplar plantations only in study area B (Fig. 2a, b). Considering

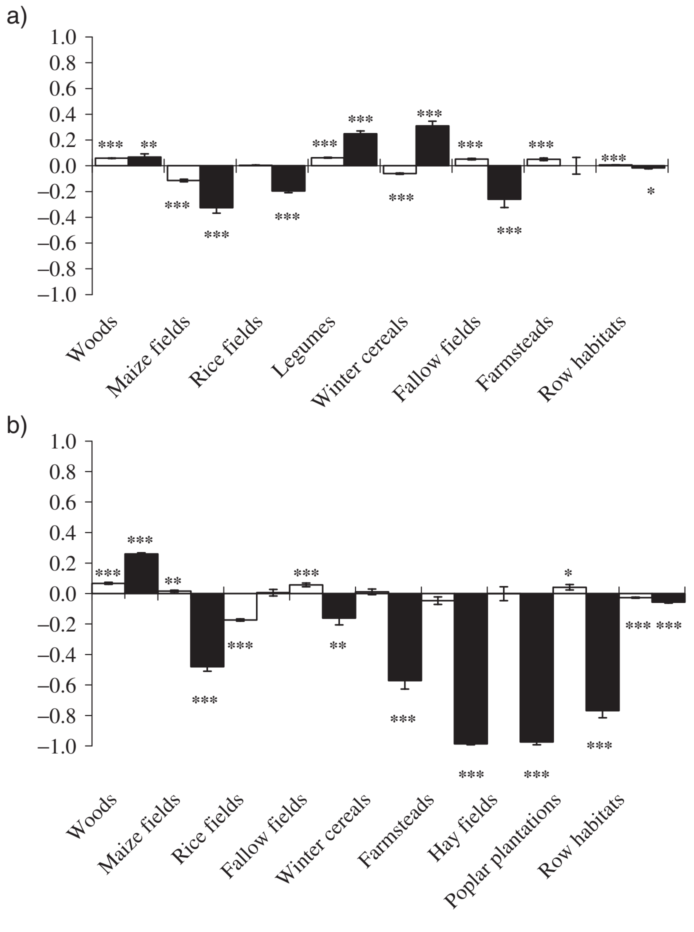

Fig. 2 Average values (SE) of Jacobs Index of preference for the use of habitat types in cottontail range defined by Kernel Analysis at 99% (*white columns*) and 50% (*black columns*) in study area A (**a**) and B (**b**) (*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$; pooled seasons)

the "core area" (distribution delineated by the kernel analysis at 50%) in study area A, woods, legumes and winter cereals were selected, whereas maize, rice, and fallow fields were avoided, and farmsteads were used as their availability (Fig. 2a). Only woods were selected in study area B, whereas the other habitat types were avoided, with the exception of rice fields that were used as their availability (Fig. 2b).

From χ^2 and Bonferroni confidence interval analyses, significant differences between the observed and expected use of habitat types were found. Fallow fields, farmsteads, and row habitats were selected in area A but only row habitats were selected in area B. Crops were generally avoided in both study areas (Table 1).

Significant differences between average values of habitat variables in plots with and without cottontails occurred in both study areas. In study area A, the percentages of woods and row habitats and the litter thickness were greater in plots with cottontails than in those without, whereas the distance from the nearest wood edge and the percentage of rice fields were greater in plots without cottontails (Table 2). In study area B, a similar pattern of the percentages of woods, row habitats, litter thickness, and of the distance from the nearest wood edge resulted; moreover the cover percentage of dead leaves was greater in plots with cottontails (Table 2).

The function derived by DFA significantly discriminated plots with pellets from those without in both study areas. In study area A, the percentage of row

Table 1 Results of Bonferroni simultaneous confidence interval analyses for the use of habitat types by cottontails (*EUP* expected usage proportion; *OUP* observed usage proportion; pooled seasons)

	Study area A		Study area B	
Habitat types	EUP	OUP	EUP	OUP
Woods	0.09	0.10	0.26	0.05**
Maize fields	0.09	0.01**	0.18	0.02**
Rice fields	0.50	0.04**	0.22	0.02**
Fallow fields	**0.01**	**0.04****	0.05	0.05
Winter cereals	0.06	0.03**	0.06	0.02**
Legumes	0.06	0.01**	-	-
Hay fields	-	-	0.02	0.00**
Poplar plantations	-	-	0.03	0.00**
Farmsteads	**0.04**	**0.09****	0.02	0.00
Row habitats	**0.15**	**0.68****	**0.17**	**0.84****
Chi-square	1467.11		564.95	
P	<0.0001		<0.0001	

** Significant differences at $P < 0.01$ (selected habitats in bold)

Table 2 Average values (SE) of habitat variables with significant differences ($P < 0.1$) between random plots with cottontail pellets (presence) and without (absence) for both study areas (one-way ANOVA; pooled season)

Habitat variables	Presence	Absence	F	P
Study area A	*N = 92*	*N = 308*		
Woods	9.6 (1.95)	5.8 (0.73)	4.85	0.028
Rice fields	11.7 (2.37)	29.4 (2.26)	16.73	<0.0001
Legumes	6.4 (1.88)	2.9 (0.65)	5.01	0.026
Winter cereals	9.7 (2.55)	5.4 (1.17)	2.87	0.091
Farmsteads	10.0 (2.70)	4.9 (0.99)	4.91	0.027
Maize stubbles	9.0 (2.22)	19.1 (1.89)	7.50	0.006
Rice stubbles	9.4 (2.47)	3.3 (0.78)	9.43	0.002
Row habitats	13.2 (0.66)	9.9 (0.36)	19.25	<0.0001
Herbaceous cover	11.4 (1.57)	7.5 (0.86)	4.89	0.028
Bushy cover	0.7 (0.24)	0.3 (0.07)	3.43	0.065
Litter thickness	0.42 (0.03)	0.28 (0.02)	14.75	<0.0001
Brightness	0.92 (0.02)	0.93 (0.00)	3.50	0.062
Distance from nearest wood edge	9.88 (1.88)	21.79 (1.61)	14.59	<0.0001
Study area B	*N = 46*	*N = 354*		
Woods	47.5 (4.69)	26.8 (1.72)	16.75	<0.0001
Rice fields	5.4 (1.73)	13.0 (1.39)	3.71	0.055
Row habitats	17.9 (1.59)	14.1 (0.60)	4.61	0.032
Herbaceous cover	20.7 (3.28)	14.9 (1.09)	3.19	0.075
Dead leave cover	4.6 (0.90)	2.4 (0.30)	6.00	0.015
Litter thickness	0.40 (0.04)	0.28 (0.02)	5.59	0.019
Distance from nearest wood edge	2.13 (0.78)	10.6 (0.96)	8.73	0.003

habitats and rice fields, the litter thickness, and the distance from the nearest wood edge were the most important variables in the discrimination, as the standardized coefficients and correlation coefficients showed (Table 3). DFA correctly classified 71.3% of original cases: 77.2% of cases with pellets and 69.5% of cases without. In study area B, the percentage of woods and row habitats, the litter thickness, and the distance from the nearest wood edge were the variables with the greatest contribution in the discrimination (Table 3). DFA correctly classified 66.0% of original cases: 71.7% of cases with pellets and 65.3% of those without.

Table 3 Results of the discriminant function analyses between random plots with and without pellets of cottontails (pooled seasons)

	Standardized discriminant function coefficients		Correlation coefficients	
Habitat variables	Area A	Area B	Area A	Area B
Woods	0.099	0.675	0.219	0.647
Rice fields	–0.221	–0.092	–0.407	–0.304
Legumes	0.412	-	0.223	-
Winter cereals	0.149	-	0.169	-
Farmsteads	0.234	-	0.221	-
Maize stubbles	0.210	-	0.306	-
Rice stubbles	–0.405	–0.173	–0.273	–0.318
Row habitats	0.353	0.485	0.437	0.339
Herbaceous cover	0.119	0.120	0.220	0.282
Bushy cover	0.065	-	0.184	-
Dead leave cover	-	0.077	-	0.387
Litter thickness	0.565	0.393	0.462	0.554
Brightness	–0.211	-	–0.179	-
Distance from nearest wood edge	–0.143	–0.075	–0.380	–0.467
Eigenvalue	0.253		0.101	
Canonical correlation	0.450		0.302	
Chi-square	88.43		37.78	
df	13		8	
P	<0.0001		<0.0001	

Discussion

Eastern cottontails were successfully introduced in both North America and Europe as a consequence of the population decline of autochthonous Lagomorphs in order to enhance game populations (Chapuis et al. 1985; Probert and Litvaitis 1996; Spagnesi 2002). The introduction success is generally linked to the dispersal ability of the species and to its attitude to colonize small patches of natural vegetation sparse in agricultural-dominated landscape (Probert and Litvaitis 1996). In Italy, the expansion of the main cottontail range occurred chiefly along the river network of the Po Plain by following the west–east direction with north–south ramifications, thus demonstrating the close relationship between cottontail presence and natural vegetation belts (Meriggi 2001).

Our analyses showed that cottontails selected woods and row habitats, including field margins and hedgerows with herbaceous, bushy and canopy cover along fields and streams in both study areas. Bruna (1952) observed that removal of bushy cover along fencerows eliminated travel lanes for cottontails and Vance (1976) indicated that reductions of fencerows caused declines among cottontail populations. Hedgerows and channel banks at the two study areas connected wooded sites and uncultivated areas. Additionally, in agricultural landscapes, row habitats could be used by cottontails for feeding in winter when the herbaceous habitats decreased (Silvano et al. 2000).

Thick layers of dead leaves and other plant litter and nearness to wood edges characterized habitats occupied by cottontails. This suggests that cottontails select habitats with dense permanent cover where there are fewer impacts from agriculture and where they can quickly find protection from predators (Linder and Hendrickson 1956). The tree canopy protects the cottontails from raptors and the dense understory makes it more difficult for many terrestrial predators to find and reach them. Friley (1955) noted that cottontails used forms located at bases of trees and shrubs. During winter, this behavior may decrease their exposure to prevailing winds, thereby facilitating homeothermy and minimizing energy expenditures (Gordon et al. 1968). Woodlots were related to the presence of cottontails in both study areas and the plots with cottontail pellets were significantly nearer to the woodlots than those without, underlining the importance of small woods for the species habitat suitability.

Habitat selection may depend partially on population density. At low density, all individuals should select the most suitable habitats, whereas at higher densities some individuals should use less suitable or suboptimal areas, culminating in use of marginally suitable areas at very high densities because of intraspecific competition (Fretwell and Lucas 1972). This pattern of habitat selection is found if the species uses one or a few habitat types or if the most suitable habitat is not available (Meriggi et al. 1992). Indeed, in study area A, where the population density was greater and natural woods were fewer than in study area B, cottontails included in the range also legumes and winter cereals in proportions greater than availability (Jacobs index) and selected (Bonferroni analysis) inhabited areas and fallow fields that may be suitable for feeding during the spring and summer because of the height of the herbaceous vegetation. Inhabited areas were made up of one big farmstead with lawns, scattered brushy cover, and ruins covered by brambles so that they may provide cover, and food and resting sites in the daytime. Among croplands, cottontails selected maize, legumes, and winter cereals, and avoided rice cultivations. Gottfried (1979) noted that wooded habitat types were conceivably isolated only in winter because cottontails probably could disperse through croplands during the growing season. We noted that crops were also used for feeding, except for rice cultivations where there was less herbaceous cover, and this is probably due to the usage of herbicides.

Eastern cottontails in Italy show the same habitat-use patterns as the species does in agriculture-dominated landscapes within its native range

(Swihart and Yahner 1984). Indeed, increasingly intensive agriculture is linked to declining cottontail populations (Edwards et al. 1981). Small patches of mixed cultivations and fragments of natural vegetation, as woods with understory and uncultivated areas connected by hedgerows with herbaceous and bushy vegetation characterize suitable habitats.

Our data also suggest that competition with native lagomorphs should be considered. European hare populations in Europe reach the highest densities in arable farmland. However, where agriculture is very intensive, hare numbers are reduced (Smith et al. 2005). Habitat diversity is positively associated with hare abundance and natural vegetation (fallow land, hedgerows, and small woods) can enhance landscape diversity, providing hares with food and cover throughout the year (Smith et al. 2005). Populations of European rabbit in Europe seem to be negatively affected by the changes in agricultural practices, in particular loss of scrubland. European rabbit density is positively associated to the ecotone that provides both food and refuge (Lombardi et al. 2003; Calvete et al. 2004; Carvalho and Gomes 2004). Competition among Lagomorph species can occur by direct interaction (interference competition), by simultaneous exploitation of limited resources (exploitation competition), as food and shelter (Pianka 1981; Probert and Litvaitis 1996), or by the ability to disperse and occupy suitable habitats. In the case of Eastern cottontails and autochthonous Lagomorphs species (*L. europaeus* and *O. cuniculus*), body size should be an important factor in winning direct aggressive interactions (Glazier and Eckert 2002). Thus there should be a dominance rank from hares, to European rabbits, to Eastern cottontails that can regulate the access to feeding patches. In fact the average weights recorded in northern Italy are 3893 g ($n = 66$, SE = 50.57; range 2,900–4,800) for hares (Meriggi et al. 2001), 1,548 g ($n = 49$, SE = 23.99; range 1,260–2,000) for rabbits (Prigioni, unpubl. data), and 839 g ($n = 20$, SE = 46.02, range 530–1,010) for Eastern cottontails (Vidus Rosin, unpubl. data). In the cottontail range in northern Italy, resources such as food and shelter are limited only during winter and, in particular, in some areas mainly cultivated with rice where hares can be forced to feed and use the field edges and the weedy vegetation along the channel banks, that are intensively used also by the Eastern cottontails. On the other hand European rabbits seem to be linked to the relict scrublands and natural broad-leaved woods. In these habitats, competition can effectively occur even if in the irrigated plain the habitat losses and the reduction of landscape diversity seem the main factors affecting hare and rabbit populations (Meriggi and Alieri 1989; Meriggi and Verri 1990). Populations of European hares and European rabbits are dramatically limited in Italy by habitat, diseases, and most important, by overhunting (mainly in the case of hares). On the contrary, hunting does not seem to be a limiting factor for cottontails that show a great dispersal ability expanding their range very fast. This attitude of Eastern cottontails is supported by the higher reproductive performance in comparison with the autochthonous Lagomorphs, in particular with hares (litter size: cottontails 5, min. 1, max. 12

- Chapman et al. 1977; Meriggi 2001; hares 2.4, min. 1, max 5 - Hansen 1992; Meriggi et al. 2001; Smith et al. 2005). In this situation, scramble competition can favor cottontails.

We need further research results in order to increase the knowledge about the competition between cottontails and autochthonous Lagomorphs; in particular, resource selection and exploitation (habitat and food) should be compared between allopatric and sympatric areas. In this way, niche shifts observed in sympatry should reflect the response to interspecific competition (Pianka 1981).

Moreover, important interactions among the three species may be through disease transmission. The results of seroepidemiological investigations, conducted in North Italy, indicate that cottontails may have a relevant role in the transmission of two of the three main diseases, myxomatosis, EBHS, and RHD, to native Lagomorphs (Lavazza et al. 2001). The high antibody prevalence found for myxomatosis confirms that cottontail is not susceptible to this disease but probably acts as a wild reservoir and plays an active role in the epidemiology of myxomatosis in rabbits, but not in hare, which is not susceptible to myxomatosis (Lavazza et al. 2001). Moreover, the results about EBHS indicated that some of the sampled cottontails could have been naturally infected by the EBHS virus developing a strong immunity, thus playing an active role in the transmission of EBHS to hares. On the contrary, from experimental infections, it seemed that cottontails are not susceptible to RHDV (Lavazza et al. 2001).

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Appendix

Appendix 1 Habitat variables measured within the 1-m radius plots and within the 100-m radius buffers

Habitat variables measured within the 1-m radius plots
Herbaceous cover (%)
Height of herbaceous cover (cm)
Bushy cover (%)
Height of bushy cover (m)
Canopy cover (%)
Height of canopy cover (m)
Dead leaves cover (%)
Litter cover (%)
Litter thickness (mm)
Brightness (ratio of the Lux measured on the point over the Lux measured in open space)
Distance from nearest wood edge (m)
Habitat variables measured within the 100-m radius buffers (%)
Woods
Maize fields
Rice fields
Fallow fields
Winter cereals
Legumes
Hay fields
Poplar plantations
Farmsteads
Row habitats

Testing Multiple Hypotheses to Identify Causes of the Decline of a Lagomorph Species: The New England Cottontail as a Case Study

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Natural History of New England Cottontails

Physical, Ecological, and Behavioral Characteristics

The New England cottontail (*Sylvilagus transitionalis*) was first described by Otram Bangs at the turn of the 19th century (Bangs 1895), and is recognized as a distinct species (Holden and Eabry 1970; Wilson 1981). Also called *coney* or *cooney*, the New England cottontail (NEC) is a medium-sized rabbit (total length: 398–439 mm, weight: 995–1,347 g; Chapman 1999) with a coat that is dark brown to buff and overlain with a blackwash that gives it a penciled effect. The anterior edges of the ears are covered with black hair and there is a black spot between the ears. These characteristics, combined with morphological features and body mass, differentiate NEC from eastern cottontails (*Sylvilagus floridanus*) (Litvaitis et al. 1991).

Historically, NEC occupied native shrublands associated with sandy soils or wetlands and regenerating forests associated with small-scale disturbances that resulted from beavers (*Castor canadensis*), lightning strikes, or local wind storms. Less frequent, but larger-scale disturbances (including hurricanes and wild fires) also provided early successional habitats, especially in close proximity to the Atlantic coast (Lorimer and White 2003). Habitats of NEC are described by vegetation structure, especially height and density, rather than specific plant associations (Eabry 1968). The most consistent characteristic of NEC habitat is dense understory cover, a feature that characterizes native shrublands and regenerating forests (Fay and Chandler 1955; Eabry 1968; Linkkila 1971). Populations quickly decline when overstory canopies close and understory vegetation becomes sparse. As a result, populations of NEC shift in space and time in response to natural disturbances and human land uses.

The diet of the NEC varies seasonally, with grasses and forbs dominating in late spring and summer (Dalke and Sime 1941). In autumn, cottontails transition to a diet of woody twigs (Pringle 1960). Barbour (1993) found limited preference for specific woody plants and suggested that the winter diet of

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NEC was largely determined by forage availability, which was ultimately determined by the size of the habitat patch a rabbit occupied.

Several investigators have studied the movements of NEC, and estimates of home range size are quite varied. Dalke (1937) used livetrapping and estimated home ranges of 0.2–0.7 ha. More recently, telemetry has revealed much larger home ranges of 2.2–7.6 ha (Goodie et al. 2003). In heterogeneous landscapes, where patches of suitable habitat vary in size, home ranges seem to be a function of the size of the habitat patch occupied by rabbits with some rabbits occupying patches as small as 0.1 ha during winter (Litvaitis, unpublished data).

When frightened, cottontails quickly flee to ground dens dug by other species or rock walls (Litvaitis, personal observation). NEC are solitary and intraspecific associations are restricted to reproduction. Dalke (1937) indicated that the testes of male cottontails begin to enlarge in late December. Pregnant females were found in wild populations from late April to August (Dalke 1937). Parturition spans late March to September (Eabry 1968), and females have two or three litters per year that average 5.2 young per litter (Dalke 1937).

Taxonomy, Distribution, and Abundance

The taxonomic status of the NEC has been revised to distinguish two sister species (Ruedas et al. 1989; Chapman et al. 1992). According to Chapman et al. (1992), populations east and north of the Hudson River have 52 chromosomes and are still identified as NEC, whereas populations west and south of the Hudson River have 46 chromosomes and are now referred to as Appalachian cottontails (*Sylvilagus obscurus*) (Chapman et al. 1992). If this reclassification is accepted, the historic range of NEC is estimated to have included most of the northeastern United States (Fig. 1).

Since 1960, the distribution and abundance of NEC has declined substantially (Johnston 1972; Jackson 1973; Litvaitis 1993). Remaining populations of NEC span <25% of their historical range (Fig. 1), and the species is currently being considered for threatened or endangered status by the US Fish and Wildlife Service (2004). Clearly, long-term viability of remaining populations is uncertain without active intervention by conservation organizations (Litvaitis and Villafuerte 1996; Litvaitis 2001).

Possible Causes of Range-Wide Decline

A number of explanations have been offered as causes of the decline of NEC populations. These can be partitioned into three major themes: a direct consequence of habitat loss and fragmentation, competition with eastern cottontails, and hybridization with expanding populations of eastern cottontails. A brief summary of each follows.

The role of habitat loss and fragmentation. Habitats within the northeastern United States have undergone dramatic changes since European colonization.

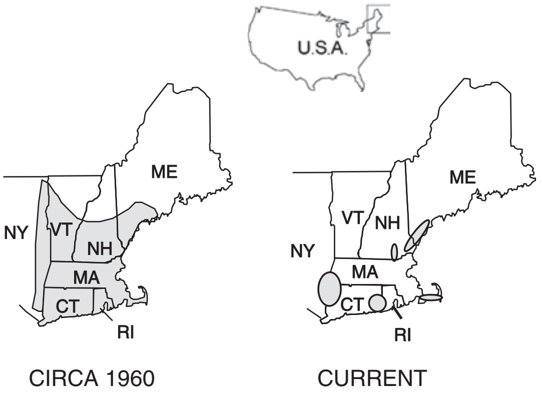

Fig. 1 Historical and current distributions of New England cottontails. Historical distribution is a compilation of Hall and Kelson (1959), Johnston (1972), and Jackson (1973). Current distribution is based on a range-wide survey of suitable habitats (Litvaitis et al. 2006). *Insert map* of the United States illustrates New England region

Initially, subsistence and then commercial agriculture resulted in large-scale clearing of native forests. However, by the late 1800s, much of the cleared land reverted to second-growth forests after farmlands were abandoned for more productive lands to the west (Litvaitis 1993). Jackson (1973) suggested that populations of NEC initially benefited from the abundance of young forests in the region during the early 20th century. According to some observers, populations of NEC were expanding at that time (Palmer 1937; Silver 1974). Nevertheless, as young forests matured, habitats became less suitable for NEC and their populations declined.

While populations of NEC were in decline, populations of eastern cottontails in the northeastern United States were increasing. Linkkila (1971) investigated the effects of habitat change on cottontail abundance in central Connecticut and reported that a mixed population of NEC and eastern cottontails changed to one that was exclusively eastern cottontails in 7 years without substantial changes in the occupied habitat. On the other hand, Chapman and Morgan (1973) suggested that the decline of forests reduced the habitats occupied by NEC and subsequent reforestation of the region created habitats that were more suitable for eastern cottontails that had been transplanted into the northeastern United States. Later, Chapman and Stauffer (1981) reported that remaining populations of NEC were largely confined to extensive forests on public lands where fragmentation was less influential.

Population decline via competition. Interference competition between expanding populations of eastern cottontails and NEC has been suggested as contributing to the decline of the latter (Fay and Chandler 1995; Reynolds 1975). Both species utilize similar habitats and foods (Dalke and Sime 1941; Linkkila 1971; Johnston 1972) and the decline of NEC populations roughly coincided with the expansion of eastern cottontail populations into large portions of the range of NEC (Fig. 2). According to the "niche width-introduction hypothesis" proposed by Chapman and Morgan (1973), the obvious range expansion and possible competitive superiority of *S. floridanus* may have been facilitated by the transplants of several subspecies (or races) of *S. floridanus* into the eastern United States. Subsequent interbreeding among these transplants may have increased the genetic variability of the offspring of transplanted eastern cottontails and functionally enabled them to occupy a broad range of habitats, including those occupied by NEC. Additionally, eastern cottontails are approximately 20% larger than NEC and therefore may be physically dominant.

Population decline via hybridization. Historically, New England cottontails were the exclusive cottontail in the northeastern United States. Prior to 1930, eastern cottontails were only detected at the extreme edge of the range of NEC (Fig. 2). Beginning in the 1920s and continuing through the 1950s, state wildlife management agencies and private hunting clubs released >200,000 eastern cottontails from mid-western populations (Johnston 1972; Chapman and Morgan 1973). These stocking efforts were an attempt to augment game populations that were declining in response to forest maturation (Litvaitis 1993). Although diploid chromosome numbers suggest reproductive isolation (NEC: 2N = 52, eastern cottontail: 2N = 42), hybridization between NEC and eastern cottontails has been reported among captive animals (Dalke 1937) and suspected of occurring in wild populations (Fay and Chandler 1955).

The reduction of a native species by hybridization has been cited as one concern of exotic introductions (Rhymer and Simberloff 1996; Simberloff 1996). Among lagomorphs, hybridization between mountain hares (*Lepus timidus*) and expanding populations of introduced European hares (*L. europaeus*) has been implicated as a cause for the regional decline of mountain hares in

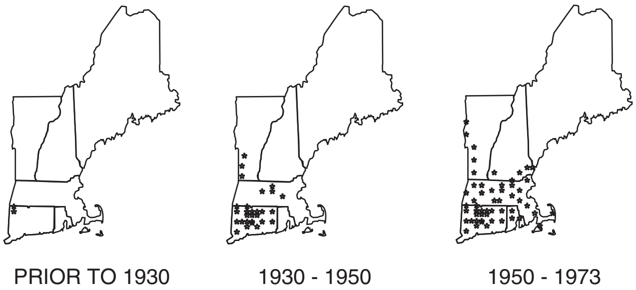

Fig. 2 Historical range expansion of eastern cottontails into the northeastern United States. Data compiled from Jackson (1973) and Johnston (1972), where each *dot* represents the collection of at least one rabbit

Sweden (Thulin 2003). Hybridization may have been especially influential in reducing populations of mountain hares where mountain hares were at low density in comparison to European hares (Thulin 2003). This scenario is similar to the situation confronted by NEC. During the 1940s and 1950s, populations of eastern cottontails were expanding rapidly as NEC populations were declining (Fay and Chandler 1955). This likely resulted in sympatric, unbalanced populations where NEC were at low densities. Therefore, it is possible that hybridization may have contributed to the decline of NEC.

Experimental Evaluation of Limiting Factors

Since 1990, we have conducted a series of investigations to test the hypotheses that have been offered to explain the range-wide decline of NEC. These projects included observational studies and manipulative experiments with free-ranging and captive rabbits.

Habitat Loss Via Forest Maturation and Fragmentation

Probably the most important disturbance that influenced the abundance of NEC was the clearing of forests for agriculture by European settlers and subsequent abandonment of these lands (Ahn et al. 2002; Hall et al. 2002). Cleared lands were abruptly abandoned in the mid-1800s for more productive farms in the mid-western United States. Many of these tracts reverted to second-growth forests (Irland 1982) and NEC and other species affiliated with early successional forests reached unprecedented levels of abundance throughout the northeastern United States in the early 1900s (DeGraaf and Miller 1996; Foster et al. 2002; Litvaitis et al. 2005). Litvaitis (1993) used information on the rate of farmland abandonment and developed a simple model of forest succession to estimate the approximate recruitment of early successional habitats. Based on the characteristics of present-day habitats occupied by NEC (especially dense understory vegetation), he assumed that cottontails occupied idle lands from 10 to 25 years after abandonment (Fig. 3). After 25 years, the second-growth forests that colonized these lands would be characterized by closed canopies with limited understory foliage (Aber 1979), and therefore, no longer suitable for New England cottontails (Barbour and Litvaitis 1993). Most of the abandoned lands matured into closed-canopy forests by 1960 (Fig. 4) and species dependent upon these habitats quickly declined (Litvaitis 1993). These results clearly indicate that the rise and fall of NEC populations was an opportunistic response to the short-term availability of early successional habitats. Predators of NEC, including bobcats (*Felis rufus*), also responded to the abundance of prey (Litvaitis et al. 2005). If populations of NEC stabilized at reduced densities, conservation actions would probably not be needed. However, early successional habitats in the northeastern United States continue to decline (Brooks 2003).

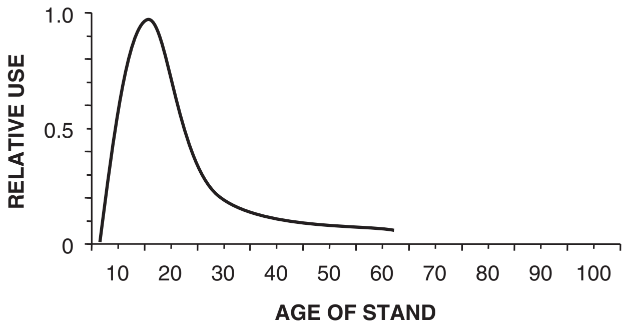

Fig. 3 Habitat association of New England cottontails in relation to forest succession that follows a catastrophic disturbance (e.g., wild fire, windthrow, or clearcut)

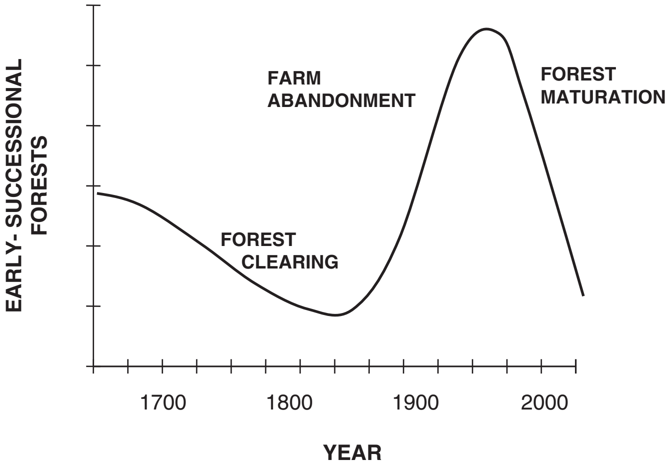

Fig. 4 Successional wave that is passing through forests in the northeastern United States following land clearing for agriculture and subsequent abandonment of these lands

In developed landscapes, the remaining populations of NEC are structured as induced metapopulations where individual patches of habitat range from <0.5 to >20 ha (Litvaitis and Villafuerte 1996).

To understand which factors are affecting contemporary populations of NEC, we conducted several field studies in southeastern New Hampshire. First, we inventoried suitable patches of habitat and found that the majority were small and often isolated. Next, we monitored habitat-use patterns and survival of rabbits on small (≤2.5 ha) and large (≥5.0 ha) patches during winter. We intentionally restricted our investigations to winter because survival during this season likely affects the ability of northern populations of lagomorphs to persist (Pease et al. 1979; Wolff 1980; Litvaitis et al. 1985). During winter, cottontails rarely ventured away from cover and preferred sites with >50,000 stem-cover units/ha [where a stem cover units/ha = deciduous understory stems + 3 (conifer stems); Litvaitis et al. (1985)]. Rabbits on small patches were predominantly males, and both sexes had lower body masses than individuals on large patches, suggesting food limitations (Barbour and Litvaitis 1993). In response to the apparent food shortage, rabbits on small patches occupied a greater variety of microhabitats, including those with limited escape cover. Such "risky behavior" resulted in a winter mortality rate among rabbits on small patches that was twice as great (0.69) as rabbits on large patches (0.35) where per-capita food resources were greater (Barbour and Litvaitis 1993).

We investigated the role of food abundance on rabbit survival by monitoring physiological condition of free-ranging rabbits using urea nitrogen:creatinine ratios obtained from urine samples collected in snow (Villafuerte et al. 1997). The nutritional plane of rabbits on small and large patches was compared to a captive population that was maintained on high-quality chow. Free-ranging rabbits on small patches started the experiment on a lower-quality diet, experienced higher mortality rates, and were killed earlier in winter than the large-patch occupants (Fig. 5a). Based on these observations, we developed a conceptual model to explain the consequences of food depletion on small and large patches. Rabbits on small patches deplete high- and moderate-quality foods earlier in winter than rabbits occupying large patches (Fig. 5b). As a result, rabbits on small patches become nutritionally stressed early in the winter and then forage in (risky) areas where they are exposed to predators and thus experience higher mortality rates than rabbits on large patches (Villafuerte et al. 1997).

These findings indicated that predation may be an important proximate factor limiting present-day populations of NEC. Because NEC likely evolved in dynamic landscapes where small-scaled disturbances were more common than those that created large patches of habitat (Lorimer and White 2003), we

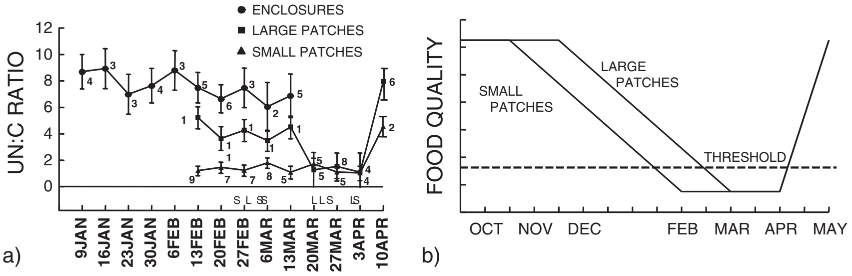

Fig. 5 **a)** Weekly mean (±1 standard error) of urinary nitrogen:creatinine ratios among captive and free-ranging cottontails that occupied large (≥5 ha) and small (≤3 ha) patches of habitat. *Numbers* indicate sample sizes and "S" and "L" indicate dates of predation of rabbits occupying small and large patches, respectively. **b)** Conceptual model of depletion of high-and moderate-quality foods on small and large patches. The threshold indicates when rabbits are likely to exhibit risky foraging behavior and become increasingly vulnerable to predation

were somewhat surprised by these results. However, our major study area (southeastern New Hampshire) and much of the region that is still occupied by NEC (Fig. 1) has been substantially modified by suburban and urban developments in recent decades (e.g., Sundquist and Stevens 1999). As a result, we suspected that contemporary land uses affect the vulnerability of NEC to predation and may also affect predators. We conducted two descriptive investigations to examine land-use influences.

First, we compared the fate (survived/killed) of a second group of transmitter-equipped cottontails to the habitat features and land uses that surrounded the patch each individual occupied. We found that the use of the surrounding land indeed influenced cottontail vulnerability to predation. Specifically, rabbits that were killed were surrounded by more developed lands (altered by removing native vegetation and adding permanent structures), more coniferous forest, and less water (i.e., rivers, lakes, and bays) than rabbits that survived the study period (Brown and Litvaitis 1995). We suspected that the abundance of disturbed lands within 0.5 km of the occupied patch had an influence on the generalist predators that were preying on marked rabbits, especially red foxes (*Vulpes vulpes*) and coyotes (*Canis latrans*).

Next, we considered how predators were responding to contemporary land uses by examining the relative abundance of predators in relation to major land uses (forest, wetland, agricultural field, pasture/brushland, and developed land) within three 54-km^2 landscapes that spanned a range of fragmentation of native forests (Oehler and Litvaitis 1996). We found that the relative abundance (based on track counts) of foxes and coyotes doubled as forest coverage decreased from 81 to 58% and agricultural plus developed lands increased from 6 to 25% of the landscape (Oehler and Litvaitis 1996). Clearly, the abundance of generalist predators, including those that prey on NEC, is enhanced by current land uses.

These results were combined with previous findings to develop a multiscaled explanation that describes the elevated risk of predation among cottontails, especially those occupying small patches of habitat (Fig. 6). At the largest scale, NEC vulnerability is a function of landscape productivity that determines predator density (Oksanen et al. 1992). In modified or developed landscapes, the distribution of life requisites (prey, water, den or roost sites) will cause predators to establish territories or concentrate their activities in certain portions of the landscape (probably multiple patches of habitat). Predators then select a specific patch to forage in according to productivity of that patch or spillover from neighboring patches with even higher productivity (Oksanen et al. 1992). Once a predator enters a patch, vulnerability is largely dependent on the microsite occupied by the rabbit. On small patches, rabbits forage in the open more often because food in cover has been depleted and they are likely more frequently exposed to predators than rabbits on large patches (Barbour and Litvaitis 1993). We concluded that the increased vulnerability to predation will cause small patches of habitat to act as demographic sinks, especially in developed landscapes.

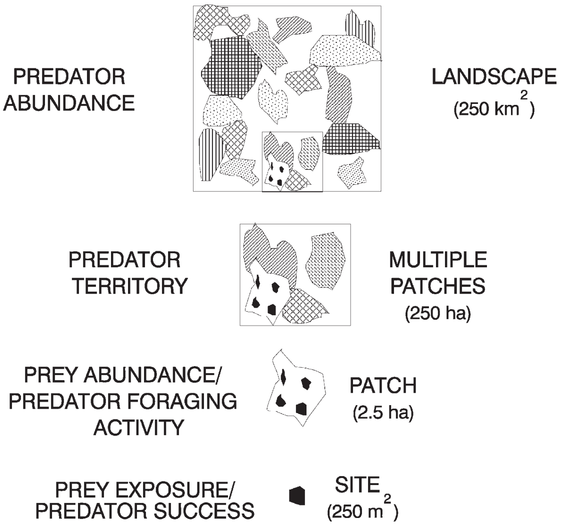

Fig. 6 Habitat attributes at four spatial scales that influence predator-prey interactions. At the largest scale, predator abundance is a function of landscape productivity. Within a landscape, individual predators establish a territory or home range by moving among multiple patches that contain all life requisites. Foraging behavior is then a consequence of prey distribution among patches within a territory. Finally, prey behavior within a patch affects vulnerability to predation

Competition with Eastern Cottontails

The ability of eastern cottontails to dominate and exclude NEC from sites that NEC occupied exclusively was evaluated by Probert and Litvaitis (1996). We examined interactions between individuals in small enclosures and also monitored use of microhabitats in a large enclosure where abundance of cover and food varied. Among behavioral dyads (a dyad is an individual of each species released into a small enclosure) where one individual dominated another, eastern cottontails were the dominant rabbit in 58% of the trials (Probert and Litvaitis 1996). This was not different from a random expectation. Additionally, no consistent differences were detected in microhabitat selection in the large enclosure. Therefore, interference competition did not seem to explain the change in distribution and abundance of NEC and eastern cottontails.

Hanski (1995) suggested that competitors may coexist in a fragmented landscape under specific circumstances. If one species is a good disperser, but a poor or equal competitor, it will become common in small patches of habitat, whereas poor dispersers (and equal or superior competitors) will dominate large patches. Under these circumstances, both species would compete more with heterospecifics, resulting in coexistence (McPeek and Holt 1992). The recent range expansion of eastern cottontails indicates that this species is a good disperser. Additionally, eastern cottontails frequently occupy habitats with limited understory cover (e.g., field edges and suburban lawns) in comparison to habitats occupied by NEC (Nugent 1968; Chapman and Litvaitis 2003). A large proportion of the habitats occupied by eastern cottontails and NEC are ephemeral, disturbance-generated habitats (e.g., idle agricultural land). If eastern cottontails were able to colonize disturbance-generated patches sooner than NEC, they may be able to maintain access to these habitats simply on a system of "prior rights", not physical dominance (Probert and Litvaitis 1996). This scenario also implies that sympatric NEC will be able to coexist with eastern cottontails if they occupy relatively stable habitats (e.g., shrub-dominated wetlands) and occupy these habitats before eastern cottontails arrive.

The differences in the ability of the two species to exploit low-cover habitats were demonstrated by a subsequent comparison of foraging strategies used by eastern cottontails and NEC (Smith and Litvaitis 2000). We monitored the response of both species in a large enclosure where cover was available in one portion of the enclosure and quality and quantity of food were varied in relation to cover. In trials where daily rations were spread among five feeders, NEC tended to stay in cover, whereas eastern cottontails sampled all feeders (Smith and Litvaitis 2000). When low-quality food was placed in cover and high-quality chow was placed away from cover, again NEC were reluctant to venture away from cover. Eastern cottontails, on the other hand, avoided low-quality food and maintained their physical condition by foraging at sites away from cover that contained high-quality food. When all food was removed from cover, NEC were reluctant to forage in the open and lost a greater proportion of body weight. Perhaps most revealing were the differences between unintended rates of predation of the two cottontails that occurred during this investigation. When food was available in cover, survivorship functions were similar, but when no food was available in cover, NEC were killed more frequently by owls and had a lower survival rate (Fig. 7).

The tendency to use open habitats by eastern cottontails could only be possible if this species had some adaptation that facilitated their behavioral differences with NEC. Smith and Litvaitis (1999) reported that eastern cottontails had a larger exposed surface area of the eyes than NEC (Fig. 8). This difference apparently enabled the eastern cottontails to detect a simulated predator (moving model of an owl) at greater distances ($\bar{x} = 21.2$ m) than NEC ($\bar{x} = 9.4$ m; Smith and Litvaitis 1999). When results of the foraging trials were applied to habitat-use patterns of free-ranging cottontails, Smith and Litvaitis (2000) estimated that NEC could only exploit 32% of the available habitat without experiencing intense predation, whereas eastern cottontails could exploit 99% of the habitats. These results indicate that the consequences of widespread forest maturation and fragmentation in the northeastern United States that have been detrimental to NEC apparently have been less deleterious to eastern cottontails. These differences in behavior and morphology probably explain the shift in composition of cottontail populations in the northeastern United States.

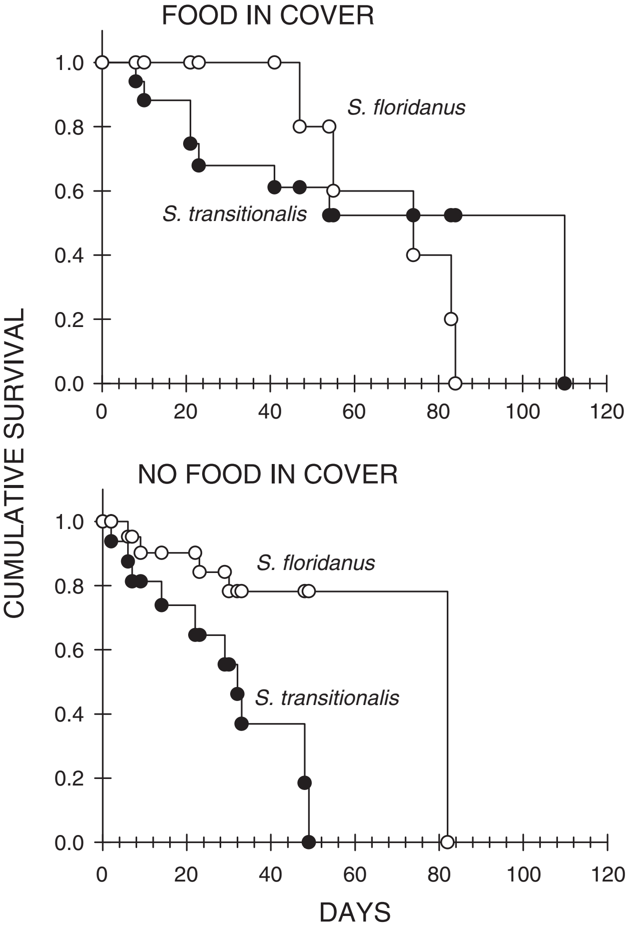

Fig. 7 Survivorship function for New England (*Sylvilagus transitionalis*) and eastern cottontails (*S. floridanus*) in a large enclosure when food was available in cover and when food in cover was removed

Hybridization with Eastern Cottontails

We used information on sequence variation in mitochondrial DNA (mtDNA) to examine the extent of hybridization between endemic NEC and invading eastern cottontails. In the eastern United States, 46 eastern, 19 New England, and six Appalachian cottontails were collected (Litvaitis et al 1997). Initial species identifications were based on skull morphology (Fay and Chandler 1955) and a discriminant model based on pelage and body size differences (Litvaitis et al. 1991). Our samples enabled us to compare populations that were never sympatric (New England versus Appalachian cottontails), recently sympatric (eastern and New England cottontails), and sympatric since the last glaciation of eastern North America (eastern and Appalachian cottontails).

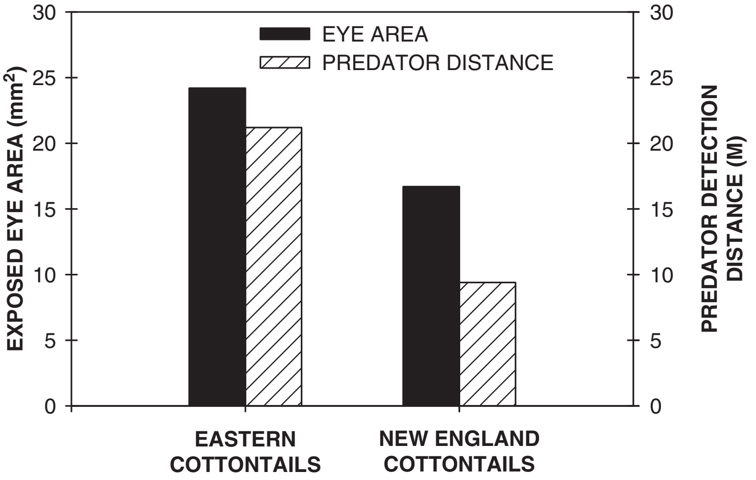

Fig. 8 Eye surface area and distance that a simulated predator was detected by eastern and New England cottontails

We found no evidence to support the occurrence of hybridization between eastern and New England cottontails or between eastern and Appalachian cottontails (Litvaitis et al. 1997). It is important to note that the maternal transmission of mtDNA may pose problems in detecting hybridization events; however, the complete lack of heterospecific haplotypes supported our conclusion that hybridization between NEC and eastern cottontails is not widespread (Lehman et al. 1991; Wayne and Jenks 1991). Our neighbor-joining tree clearly identified two distinct and separate clusters for the two species (Litvaitis et al. 1997). This separation was further supported by morphology, and by complete agreement between identifications based on morphology and mitochondrial haplotypes (Litvaitis et al. 1997). Surprisingly, a phylogeographic analysis did not reveal separation between New England and Appalachian cottontails (see Litvaitis et al. (1997) for an explanation).

Remnant Populations of New England Cottontails

Recently, we completed a range-wide survey of habitats occupied by NEC largely based on fecal pellet collections (Kovach et al. 2003). Results suggest that the remaining NEC are restricted to distinct populations (Fig. 1). A preliminary investigation of genetic variation based on microsatellites of nuclear DNA from 82 individuals sampled across the current range of NEC supported these separations (Kovach et al., unpublished data). Rabbits collected in eastern Massachusetts diverged from all other subpopulations, largely due to the near homogeneity of the samples tested. These results may indicate a genetically depauperate population as a consequence of isolation. Additional efforts on this topic are warranted, including a comparison with historically collected specimens to determine if current geographic structure of genetic variation is a recent consequence of population decline and isolation. Such information may be especially relevant if translocations are used to establish additional populations in vacant habitats.

At the landscape scale, local populations seem to be connected by anthropogenic features that may facilitate dispersal (e.g., brushy margins of major roads and railroad corridors). These features may be especially important in portions of the range of NEC where human settlements and dense road networks have fragmented suitable habitats. In southwestern Maine, for example, landscapes surrounding patches occupied by NEC had a greater abundance of old fields, roads in classes 1 and 2, and total roads and less forest than the landscape surrounding vacant patches (Litvaitis et al. 2003). The apparent influence of brushy roadsides also included the largest road in the area, Interstate Highway 95. We encountered extensive stretches of Interstate 95 that had shrub-dominated margins >10 m wide. Additionally, rest areas and exit ramps had sufficient disturbance-generated habitats to support 1–2 cottontails (J. Litvaitis, personal observation). Bolger et al. (2001) also found that the vegetated edges of highway rights-of-way were frequently used by a variety of small mammals, and these served as movement corridors between disjunct habitat patches. Although Interstate 95 poses a formidable barrier (up to seven or eight lanes of vehicle traffic) to east–west movement, the substantial habitat associated with this corridor may facilitate north–south movement. In southern New Hampshire, we found that one of the most expansive populations was associated with railroad corridors (J. Tash et al. unpublished data; Fig. 9). Here, the strip of brushy vegetation also may be functioning as an important dispersal corridor.

Based on these results, we recommend that efforts to enhance existing populations of NEC and possible translocations consider habitat management in a landscape context. We recommend two basic approaches.

Management in Fragmented Landscapes

Efforts to maintain thicket habitats in more developed landscapes are confronted with a variety of limitations and challenges. Remaining habitats occupied by NEC are often fragmented by dense road networks and suburban developments (Litvaitis et al. 1999; Litvaitis et al. 2003) and NEC are often spatially structured as induced metapopulations (Litvaitis and Villafuerte 1996). In such an arrangement, small populations are able to persist only because surplus rabbits from one or more large populations regularly disperse to small patches of habitat. In these circumstances, it may be most effective to establish and maintain moderate (>10 ha) to large size (>25 ha) patches that can serve as “core habitats” (Litvaitis 2001). In some instances, it may be possible to develop degraded sites (such as abandoned gravel and

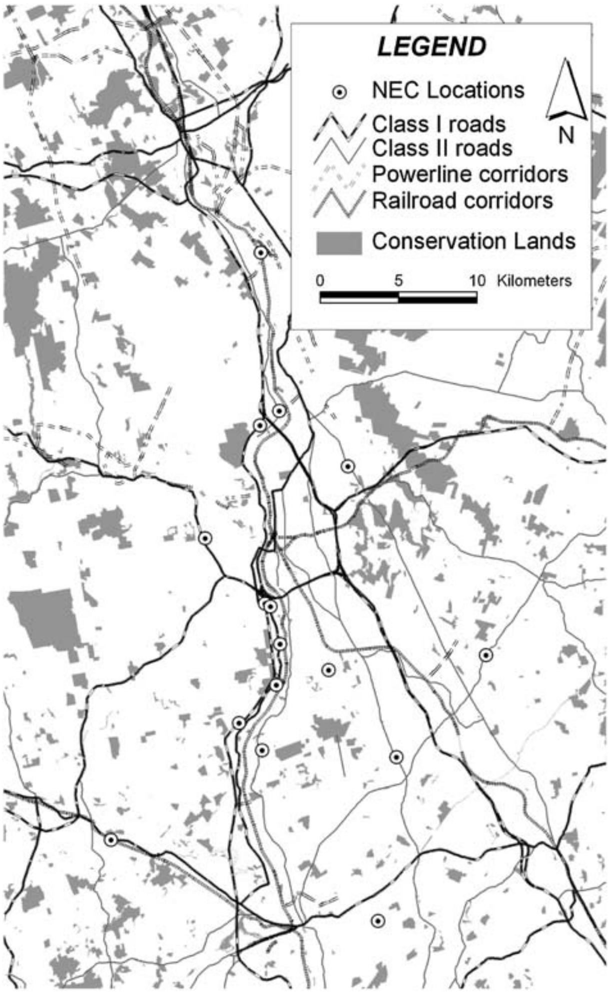

Fig. 9 Distribution of patches occupied by New England cottontails in southern New Hampshire. Note that most occupied sites are in close proximity to a railroad or road rights-of-way. The brushy edges of these corridors may facilitate dispersal of cottontails. Existing populations are near to but do not currently occupy conservation lands

sand mines) into core habitats with only modest initial effort and periodic maintenance. These tracts could support populations of small mammals and passerines that would be less susceptible to the limitations of the surrounding landscape matrix and large enough to withstand short-term perturbations (Litvaitis et al. 1999). Clustering core habitats and placing them adjacent to existing land-uses that include early successional habitats might facilitate exchanges among neighboring populations (e.g., utility corridors). These narrow landscape elements may not function as source habitat for NEC because red-tailed hawks (*Buteo jamaicensis*) and other raptors often select these as foraging and nesting habitat (Denoncour 1982), but they may serve as dispersal corridors and they do provide suitable reproductive habitat for a number of songbirds (Askins 1994; Confer and Pascoe 2003). However, there is an important caveat associated with managing habitats near dense road networks. Specifically, such habitats near roadways may attract predators of NEC and result in vehicle collisions. Therefore, we suggest that core habitats be positioned away from dense road networks to avoid creating an ecological trap for predators (Litvaitis et al. 1996; Litvaitis 2001).

Management in Relatively Intact Landscapes

In less-developed regions of the northeastern United States, habitat-management programs that resemble natural disturbance regimes may be appropriate (Seymour et al. 2002). Where feasible, restoration of native shrublands should be a management priority. In regions where the historic abundance of shrublands and barrens was limited, timber harvests will provide a practical approach to diversify stand age distributions. Here, a “sliding scale” approach would be appropriate in mid-successional forests where natural disturbances are rare (Litvaitis 2003). Initially, the size of timber harvests would be larger than natural disturbances to offset the shortfall in early successional habitat that currently exists. Once established, some of these openings could be maintained by active management (e.g., cutting, mowing, or control fires). As forests mature, management efforts (especially timber harvests) could then be patterned after canopy gaps (Runkle 1991) or modified to specific silvicultural practices of a region (Seymour et al. 2002) if other forms of NEC-suitable habitats (e.g., native shrublands and beaver impoundments) are adequately represented. Such an approach may be most appropriate on public lands or industrial forests where road networks and elevated populations of generalist predators may not be a concern.

Conclusions

Our research has identified factors that contributed to the historical rise and fall of NEC populations throughout the northeastern United States. This species is now confronting substantially altered landscapes where current land uses continue to reduce the abundance and connectivity of suitable habitats. Additionally, generalist predators in many of these modified landscapes are probably more abundant than ever before. As a result, active intervention is essential if this species is to persist. Initially, management efforts should be directed at expanding existing populations that occupy habitats where eastern cottontails are absent. Maintaining and establishing populations of NEC that are sympatric with eastern cottontails will be challenging, yet such efforts are warranted if NEC are to function as more than a taxonomic novelty.

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Physiology and Behaviour

Many Common Odour Cues and (at Least) One Pheromone Shaping the Behaviour of Young Rabbits

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Introduction

Rabbits develop through a succession of periods delimited by the rate and quality of mother–young interactions, the nature of ingested nutrients, and the level of physiological and behavioural autonomy. Two transition periods concentrate peak challenges to the viability of young rabbits and recruit all their adaptive resources: the days after birth, and the days around weaning[1].

The neonatal mortality peak may reflect difficulties in acquiring milk. Newborn pups are then constrained to contact the mother, find nipples, and suck within the 3–5 min the female makes herself available during the day (a regimen lasting for the first 2 weeks postpartum; Zarrow et al. 1965; Lincoln et al. 1974; Selzer et al. 2004). Further, the number of littermates aiming at milk (ranging from 6 to 15 in breeding conditions) often exceeds offered nipples (8 to 10 depending on breeds), inducing intense sibling rivalry (Drummond et al. 2000). But as individual pups do not monopolize a nipple during the same nursing bout (Drewett et al. 1982; Hudson and Distel 1983; Bautista et al. 2005), all valid pups can in principle come to the milk resource[2].

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[1] Apart some works of Mykytowycz (e.g., 1985) and of several other field biologists, most observations of parental/filial behaviour in the rabbit are derived from domestic breeds. Thus, the validity of these data may be restricted to the sheltered contexts in which they were gathered, although several studies show conserved behaviour patterns in wild and breeding conditions (e.g., Deutsch 1957; Kraft 1979; Broekhhuizen and Mulder 1983; Hoy and Selzer 2002; Selzer et al. 2004).

[2] It is not rare that one or two pups per litter miss suckling at one feed. Older pups can even miss two consecutive feeds. This is apparently inconsequential although the initial success of colostrum intake conditions later viability (Coureaud et al. 2000a).

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In addition, the sucking success of individual pups improves from day to day through the fine-tuning of directional cues, the training of motor skills and the gain in body weight. Body weight is highly protective from occasional inanition (Coureaud et al. 2000b; Drummond et al. 2000) and depends on the realization of efficient strategies to convert milk into biological tissues, based on huddling within the litter and optimising of the insulating properties of the nest (cf. Hudson and Distel 1982; Jezierski et al. 1997; Bautista et al. 2003).

The second mortality wave of young rabbits corresponds to the transition from exclusive dependence on milk to the post-lacteal diet. By the end of week 2, pups progressively exit the nest to initiate nursing and stay then close to the female for increasingly longer periods (e.g., Scapinello et al. 1999). With the elevation of energetic needs, pups turn to non-milk resources, first in ingesting small quantities of maternal faecal pellets and nest material, and then in adopting the adult diet. After postpartum day 20, the milk yield of rabbit females decreases progressively (Cowie 1969; McNitt and Moody 1988), and nursing ceases more or less abruptly (depending on ongoing pregnancy; Gonzalez-Mariscal and Rosenblatt 1996). Pups are then seen feeding beside the mother or littermates, and they are progressively enforced to ingest solid foods as their sole diet (Scapinello et al. 1999). So far, little is known about when exactly pups initiate caecotrophy, the other major source of nutrients in post-lacteal diet (see Gidenne and Lebas 1987; Orengo and Gidenne 2005).

Along these early transitions, the behaviour of young rabbits is predominantly organized by somesthesis and olfaction, at least during the period when they are functionally deaf and blind (until days 7 (Volokhov 1968) and 9–16 (Warkentin 1937), respectively). Olfaction mediates nipple localization and milk intake, and later social integration. From early on, pups develop differential responses to odours from their mother's skin gland secretions (Mykytowycz and Ward 1971). It has become clear from research conducted in other mammalian infants that learning, and specifically odour learning, is fundamental in early adaptive development (e.g., Rosenblatt 1983; Alberts 1987; Hudson et al. 1999a; Schaal 2005). Rabbit pups are no exception, and it is the aim of the present chapter to survey research on the early functions of odours and learning. Pups indeed rely on a variety of information means to find their way to nipples. Some of this information results from evolved signalling processes between emitting females and receiving pups, whereas others are tuned by environmental circumstances during (prenatal or postnatal) development. *Signals*, and among them *pheromones*, pertain to the former category, whereas *common odour cues* variably transmitted by females to pups belong to the latter category. Recent data on these two kinds of olfactory influences used by rabbit females to optimise offspring adaptation are summarised below.

Olfaction-Guided Behaviour in Developmental Transitions

Interfering with the ability to process odours clearly illustrates the importance of olfaction for rabbit pups. Newborns rendered anosmic by olfactory bulb lesion or by peripheral inactivation of the olfactory mucosa loose their ability to locate a nipple and starve to death (Schley 1977, 1979). Further, if the normal abdominal odour cues of a lactating rabbit are washed off, the pups are delayed in finding nipples (Müller 1978). We will emphasize in this section how neonatal rabbits are able to extract and store olfactory information at the different stages of development, and to translate it into adaptive behaviour.

Chemosensory Continuity between Foetal and Neonatal Niches

Rabbit pups are not born into a completely unknown environment. Placentae stain the nest material (when they are not all immediately eaten by the doe; Hudson et al. 1999b; Melo and Gonzalez-Mariscal 2003), amniotic traces and foetal/maternal blood visibly taint the nest and littermates, and it may be assumed that corresponding odour traces are spread on the abdominal fur by the female's occasional self-licking activity during and after parturition (Hudson et al. 1999b), and by newly born pups themselves rooting through the mother's fur. Rabbit pups show positive responses to the odours of freshly collected placentae (although they do apparently not to amniotic fluid; Coureaud et al. 2002). Empirical evidence also exists about pup attraction toward colostrum and milk (Müller 1978; Keil et al. 1990; Coureaud et al. 2001a, 2002). Thus, the substrates that contact the nasal chemoreceptors in foetal or neonatal pups elicit attraction.

When introduced into a test arena containing simultaneously the odours of fresh placentae and of rabbit milk, newborn pups orient randomly (Coureaud et al. 2002). This non-selective response may reflect equivalent sensory treatment the two odour stimuli (Schaal 2005). Several processes may cause odour similarity between perinatal fluids. One of these processes is based on the passage of odorous compounds from the female's diet into the amnion: for example, rabbit pups born to does fed juniper, thyme, or cumin seeds subsequently prefer such odorants in spatial or food preference tests (Semke et al. 1995; Coureaud et al. 2002). Foetal rabbits have thus the capacity to perceive an odour encountered in the amniotic fluid, and then, as neonates, remain able to pick out this odour in the postnatal environment, and especially in milk (Coureaud et al. 2002).

The adaptive value of the chemosensory similarity between amniotic fluid and colostrum/milk is clearly revealed by its perturbation (Coureaud et al. 2002). When, in an experiment conducted right after delivery, half of each litter is cross-fostered to a female that had eaten the same food as the biological mother, the other half being given to a female fed an olfactorily distinct regimen, pups exposed to perinatal continuity (so-called 'continuous') and pups exposed to perinatal discontinuity ('discontinuous') differ in sucking success and amount of milk consumed. 'Continuous' pups have higher sucking success than 'discontinuous' pups on the first two sucking bouts. In addition, those who accede to nipples in the 'discontinuous' group ingest less milk than the successful pups in the 'continuous' group. Thus, the alignment of the actual olfactory properties of milk (or of mother's belly) with the olfactory experience encoded in utero influences the efficiency of initial sucking performance in rabbit pups.

Weaning

Prenatal memories can subsist for longer periods than the first days to influence food choice at weaning: rabbit does having eating during pregnancy and lactation either standard lab chow, or chow mixed with juniper berries or thyme, produce pups that will prefer correspondingly odorised food at weaning (Altbäcker et al. 1995). The effect of odour exposure in utero, in milk, or in faeces on food intake has been assessed in groups of 28-day-old pups exposed to juniper through either (i) amniotic fluid + milk + faecal pellets, (ii) amniotic fluid + milk (iii) amniotic fluid only, (iv) milk only, (v) (hard) faecal pellets only, and (vi) no exposure at all (Bilko et al. 1994). Interestingly, the groups having encountered juniper odour through any medium showed equally strong appetence for juniper berries as compared to control pups. Rabbit pups can thus acquire odour information from their mother's diet at any stage of development. This overlap in the ways of doe-to-pup transmission of odour cues certainly favours the early encoding of odorants that predominated in the mother's diet and which were not harmful to her. How such mother-induced odour experience modulates the initial feeding trials in weanlings and the establishment of their dietary profile remains to be investigated both in captive conditions when the animals are provided with a controlled variety of foods or in the wild when they have access to the complex food environment surrounding the nest entrance.

As suggested in the above experiment, maternal faecal pellets may be significant in passing odour information to pups about food. From postpartum day 3–4 until about the end of the second week, females indeed drop a small amount of hard pellets in the nest after each nursing bout (Mykytowycz and Ward 1971; Hudson et al. 1996). These pellets are very actively nibbled and finally consumed by the pups (Moncomble et al. 2004; Moncomble 2006). The functional impact on pups of eating the hard faeces left in the nest may go beyond mere food-related information conveyance. Additional advantages can be suggested partly based on comparative evidence (e.g., Moltz and Lee 1981): (1) Faecal pellets may also provide information related to adult colony members' identity due to the fact that they are coated with secretions from the anal glands (Mykytowycz 1985). (2) Contact with maternal faecal pellets may promote the rapid, massive transfer of non-pathogenic gut microflora and the accompanying boost in digestive mucosal immunity (Padhila et al. 1995; Fortun-Lamothe and Boullier 2004). (3) Ingestion of solid matter may favour the inception of caecal fermentation and the maturation of caecal peristalsis leading to the dual excretion of hard/soft-faeces. Finally, (4) maternal faeces provide nutritious substrates (Gidenne and Lebas 1987). It is well known that preventing adult rabbits from ingesting faeces is detrimental to their health (e.g., Olsen and Madsen 1944), but so far the consequences of preventing pups from ingesting maternal faeces has not been systematically investigated, although evidence exists that neonatal consumption of maternal droppings accelerates colonization by non-pathogenic gut bacteria (Kovacs et al. 2004, but see McNitt and Moody 1987).

Opportunistic Odour Learning: Early Plasticity of Significant Odour Cues

Learning Odours in the Nest

In the initial period following birth, the nest is composed of plant materials[3] and fur self-plucked by the female from her ventrum. After delivery, the nest may be dominated by odorous substrates derived from the female (fur, faeces), or selected by the female from the environment (plant material). From female-dominated, the chemical ecology of the nest may shift to pup-dominated. Over the first weeks, the nest changes from a fluffy lining into which pups dig and cover into a packed, strong-smelling mattress soaked with pup urine. The materials composing the nest are olfactorily salient to newly born pups. When subjected to preference tests for nest materials or control stimuli, pups display preference for the former after minimal or no prior postnatal exposure (Hudson et al. 2003). Pups can also show attraction to an artificial odorant applied in the nest (Hudson 1993). In sum, pups are sensitive to the odour properties of the nest, and such very early responsiveness may be related to learning right after birth or even to the prior exposure to the odour of plants eaten by the pregnant female and concurrently introduced into the nest lining.

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[3] The plant species chosen by females as nest materials have not been described in detail, although it is known that a great variety of plants is browsed (e.g., Chapuis 1979; Kertesz et al. 1993). It would be interesting to verify whether, for building their nests, does select plants carrying odorous compounds known to repel parasites as has been noted in birds (e.g., Lafuma et al. 2001; Petit et al. 2002).

Learning Odours While Suckling

Although pups are obviously capable of locating nipples from the very first minutes following birth (Schley 1979; Hudson 1985; Coureaud et al. 2000c), based on odour signals that will be described below, the sensory control of nipple searching and sucking by rabbit pups is fine-tuned by experience. This is highlighted by the fact that newborn rabbits become increasingly skilled in attaching to nipples over the first days in terms of latency to latch on or to switch between nipples (Müller 1978; Drewett et al. 1982; Hudson and Distel 1983). This amelioration may be related to the general maturational improvement in olfaction or somesthesis, to the learning of critical odorants around the nipple, to the iterative execution of motor patterns related to rooting-sucking activities, or (most probably) to all three processes combined.

Rabbit pups are remarkably fast in learning odours associated with suckling. An important series of studies looked for the sensory cues and the reinforcing processes that *could* come to control searching and oral seizing of nipples in newborn rabbits. The rabbit was pioneered in the 1950s by Russian investigators as a model for early odour learning. Among others, Ivanitskii (1962) described the precocious learning of artificial odorants (e.g., "Chypre" cologne, oil of camphor) painted on the mother's ventral fur. By re-presenting these same odorants outside the nursing context on the experimenter's own hand, evidence was then provided that from the 1st day after birth rabbit pups can learn in only one session a non-specific odorant associated with searching-sucking activity. Ivanitskii also showed a developmental change in the pups' propensity to associate an odorant with sucking, as learning was easiest to establish on days 1–10 than on later days. These data were further worked through by Hudson and her collaborators (Hudson 1985; Kindermann et al. 1994; Allingham et al. 1999). In their basic procedure, these investigators established learning by scenting a doe's ventral fur with varied odorants (e.g., citral, camphor, Chanel n°5, or garlic), letting pups search and suck on such a scented doe, and thereafter testing them on different surrogates (such as similarly scented vs. control does or pieces of fur). It came out that pups express the typical sequence of nipple search and attachment on an unfamiliar doe or even an anesthetized cat painted with the same odorants after only one nursing-odour pairing. This learning was selective as odourless controls as well as surrogates scented with different odorants were ineffective in conditioned pups. In addition, pups that were not exposed to the nursing + odour pairing did not respond by nipple attachment to the experimental odorants on the surrogates. This robust *one-nursing odour learning* led to a series of studies unravelling same underlying processes.

It appeared first that nursing-induced learning develops progressively after the conditioning trial, full response expression being delayed until 8–16 h after the associative episode. A consolidation period is thus necessary before the conditioned stimulus can affect the full expression of searching-grasping (Hudson 1993). This means that odour learning during a previous suckling episode prepares effective cues for the next suckling episode. But, in addition, such learned odorants can remain active for several days after conditioning. Pups conditioned on an artificial odorant on day 1 and then deprived of it in subsequent suckling, can still fully express the typical searching behaviour when put on a similarly scented female on day 5 (Hudson 1985).

Second, Hudson et al.'s work corroborated Ivanistkii's observation on the time-bound character of odour conditionability in rabbit pups. The one-session learning of the odour associated with sucking a nipple is only effective during the first 4 days after birth (Kindermann et al. 1994). After day 5, the nursing-induced odour learning vanishes completely, unless the pups are deprived of natural suckling experience (by bottle-feeding), which then prolongs their conditionability to an artificial odorant for several additional days (Kindermann et al. 1994). This result raises the possibility that the first 4 days after birth represent a special stage for acquiring odours contingently with suckling.

Third, the reinforcing processes contributing to nursing-induced odour learning were assessed in controlling the most obvious reinforcers afforded by a lactating doe: milk intake and the possibility to perform searching-sucking (Hudson et al. 2002). The efficacy of learning a novel odorant was compared in groups of pups exposed to a scented doe while being allowed to express: (i) normal searching and suckling behaviour ('search-suck-milk' group), (ii) normal suckling without getting milk ('search-suck' group), (iii) searching without sucking ('search' group), and (iv) forced sucking without prior searching ('suck' group). When tested 24 h later on a rabbit fur scented with the conditioned odorant, pups of groups i–iii expressed the same level of searching. As the milk condition (group i) and the non-milk conditions (groups ii–iii) did not differentially affect searching in the test, milk intake per se was interpreted to be inefficient in the establishment of nursing-induced odour learning. The mere expression of searching on a lactating female's fur was also ineffective, but the realization of sucking revealed to be the strongest factor. These results were interpreted as evidence that sucking-related intra-oral stimulation, rather than contact with and ingestion of milk, was the key-reinforcing agent that leads to odour learning in the context of searching on an anesthetized doe's abdomen (Hudson et al. 2002). Although sucking may improve the strength of learning in the conditions of the above studies, the realization of sucking during the conditioning procedure was unavoidably confounded with the exposure to nipple odour. Thus, an alternative hypothesis would be that a potent, non-obvious factor associated with the nipple could be the key-agent in early odour conditioning.

It becomes clear from the above results that the context, and the act itself, of sucking are efficient promoters of odour learning in rabbit newborns, as they are in other mammalian newborns (e.g., Blass and Teicher 1980; Nowak et al. 1997). An arbitrary odour cue present on the nipple surface can be assigned incentive value during a single suckling episode, and it can remain active during the next suckling episode, 24 h later or more. Nursing-induced learning of odours may thus be a process by which pups can update the properties of milk, which are known to change from day to day (Cowie 1969; Hall 1971; Lebas 1971), and therefore they can anticipate odour-based changes in the mothers' conditions (e.g., variations in chemosensory properties of milk as a function of shifts in the doe's diet or lactational physiology).

Learning Post-Lacteal Foods

The ways and rate of the pups' acquisition of odour information from the womb, milk, ventral fur, and faeces have already been sketched above. These results follow several mammalian principles in the transfer of odour information from mother to offspring (Schaal 2005; Schaal et al. 2005). First, a principle of chemical continuity applies: odour compounds create a thread of familiarity that newborns can trace while experiencing a background of novelty in space, food, or social partners. As mentioned above, odour cues reflecting the mother's diet are present in the amnion, milk, nest and faecal pellets; they do also stain the mouth and breath of the female while she eats, and finally they are emitted from solid foods encountered in the mother's presence. A second functional principle connects such perinatal chemical continuity with chemosensory plasticity. The pervasive presence of given odour cues during development interacts with the neural and perceptual organisation of the pups. Rabbit pups may be first primed to certain odour cues in utero; such cues are confirmed as incentives by their association with postnatal reinforcers (sucking, milk), and then become established into stable 'search images' that govern motivated behaviour in growing pups. Rearing experiments in which such odour cues are selectively controlled at given stages of development reveal that pups can integrate them at any stage and retain them in the long run (Schaal 2005; Schaal et al. 2005). Sensitive periods for facilitated odour learning may temporarily bias sensory abilities, and subsequently give stronger significance to certain stimuli (Ivanistkii 1962; Kindermann et al. 1994). Thus, the proficiency of rabbit pups in learning the odour of almost any substance filtered out as 'non harmful' or 'beneficial' by the mother, and to use them as predictable cues in the next developmental niche, reflects processes of individual self-organisation in the ever changing environment.

A Species-Specific Signal Developing without Learning

Individual-Specific Versus Species-Specific Odour Cues

Rabbit pups were shown above to display greater preference for a milk which is olfactorily aligned with the dominant odour experienced in utero, i.e., pups born to cumin-fed does seize more readily a glass-stick dipped in milk from cumin-fed females, while pups of does fed standard food more readily grasp a stick dipped in the milk from females receiving a standard diet (Coureaud et al. 2002a). It was remarkable, however, that these pups responded at a much higher rate to either milk than to the control stimulus (water), indicating that rabbit milk releases oral grasping regardless of its aroma content. This suggests that pups can attend to several odour cues carried in rabbit milk, some reflecting individual characteristics of the mother (e.g., her diet), others representing higher-level meanings, related to population, genus, or species.

Species-specific cues were studied either by presenting 'naïve'[4] pups with given rabbit substrates from unrelated females, by exposing them to homologous compounds from other species, or conversely by presenting rabbit substrates to newborns of other species. From such experiments it became clear that female rabbits and their milk are particularly potent in releasing head searching and oral grasping in pups. Rabbit pups do not respond in such a way when put on anaesthetized lactating rats, cats, or hares (Müller 1978; Hudson 1985), but when put in contact with (involving tactile, and volatile and non-volatile chemostimulations; Hudson and Distel 1984, 1990) or above the abdomen of rabbits (involving only volatile chemostimulations; Coureaud and Schaal 2000), pups do respond discriminatively to males, virgin, or lactating females. The latter release the strongest orientation behaviour and they do so in early, rather than late, lactation, and in the pre-nursing, rather than the post-nursing, phase (Coureaud et al. 2001). Some efficient volatile factor(s) appear thus to be tightly linked with lactation. Whether the test female is the mother or an unfamiliar female fed the same diet does not matter (Müller 1978; Hudson and Distel 1984), indicating that lactating females emit invariant signal(s) which attractive power override(s) individual-specific cues. This(ese) olfactory factor(s) appear(s) to be released from the lactating females' abdomen, and specifically the nipples (Müller 1978; Hudson and Distel 1983; Coureaud et al. 2001; Moncomble et al. 2005).

Further, the abdominal odour of lactating females is as attractive as the odour of fresh rabbit milk in a test opposing them simultaneously (Coureaud et al. 2001), showing that particularly active compounds are produced in, on, or around the nipple, from where they can perhaps be simultaneously emitted in air and in milk. Müller (1978) evidenced that rabbit milk odour is unique in eliciting the responses of newborn rabbits (bovine, ovine, porcine, and feline milk is completely inert). Further, this author found that the releasing potency of rabbit milk is rapidly lost after milking, a finding later corroborated by Keil et al. (1990) and Schaal et al. (2003). Thus, all evidence converged to designate milk as the substrate that carries a potent odorous factor[5] eliciting orientation,

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[4] "Naivety" is operationalized here by testing newborns before extensive postnatal exposure to, or by depriving them of, the target stimulus before the test.

[5] In the context of the eager quest for the identity of the volatile factors releasing suckling behaviour in newborn mammals (e.g., Ewer 1961; Hofer et al. 1976; Teicher and Blass 1976, 1977; Rosenblatt 1983; Vince and Ward 1984), this species-specific attraction effect of odorants from

rooting and nipple attachment in rabbit pups. It is not excluded that other substrates (e.g., abdominal skin gland secretions) also carry active compounds [as pregnant females (Hudson and Distel 1984; Gonzalez-Mariscal et al. 1994) and even males (Schaal and Coureaud, personal observations) can elicit searching responses in pups], but the precise source remains to be established. Milk being the handiest source of the potent sucking releaser, all subsequent analyses have been directed on it.

A Species-Specific Factor in Rabbit Milk

From the physical, chemical, and biochemical standpoints, milk is a multifaceted mixture. A first step in reducing this complexity was to select the volatiles of the headspace developing over milk when it stands for a standardized time. However, in such conditions the chromatographic profile of rabbit milk volatiles remains exquisitely complex, with more than 150 peaks on gas chromatographic (GC) tracings. A way had thus to be found to further reduce the complexity of milk in pinpointing the GC peaks which correspond to the highest behavioural impact. To that aim, a gas chromatography-olfaction (GCO) test was developed for rabbit pups. This approach made possible the separation of the milk volatiles that are detected simultaneously by rabbit pups held to the sniffing port of the GC and by the flame ionisation detector (Schaal et al. 2003). After 25 such GCO tests, the figure of the most reactogenic regions of the chromatogram was obtained: 21 peaks directly linked with more acute pup responsiveness were identified by GC-mass spectrometry, and the corresponding 21 compounds presented to pups on glass sticks. Among these 21 candidates, a single compound, 2-methyl-but-2-enal (2MB2), was found to be the most effective (Schaal et al. 2003; Coureaud et al. 2003).

To corroborate the effect of 2MB2 in milk, its concentration in the headspace was dosed in parallel with the behavioural activity of milk left at ambient temperature. Recall that the behavioural activity of milk then drops drastically. Thus, if 2MB2 contributes to the activity of milk, any decrease of milk

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the mammary field in the rabbit was first named by Schley (1976) “Geruchskomplex der Bauchhaare” (p. 150), or “Pheromon postulierten Geruchskomponente” (p. 147), to merely end with “Pheromon” (Schley 1977). The same author later took care to return to the term “mütterlicher Geruchsstoff” (Schley 1985). The term ‘pheromone’ remained however in use to label the behavioural response of pups to undefined odour cues released from the abdominal surface of the doe or from milk (e.g., Müller 1978; “nipple-search pheromone”: Hudson and Distel 1983, or “nipple pheromone”: Gonzalez-Mariscal et al. 1994) although it has been originally coined to define a class of biologically active *substances* (Karlson and Lüscher 1959), not merely their *effects*. But this terminology stimulated abundant investigation on the physiological conditions for lactating females to release optimally nipple attachment in pups (reviewed in Hudson and Distel 1995; Gonzalez-Mariscal and Rosenblatt 1996). This corpus of research was obviously a prerequisite for the chemical identification of the maternal compound bearing special behavioural activity for rabbit pups.

activity should correlate with a fall in 2MB2 content. The concentration of 2MB2 dropped drastically after 30 min of milk exposure to air, indicating that its content parallels the releasing potency of milk (Schaal et al. 2003). In addition, when fresh rabbit milk is inactivated by bubbling nitrogen through it, adding 2MB2 in adequate concentration reinstates its full behavioural effect. Thus, 2MB2 clearly is *one* key-factor responsible for the behavioural activity of rabbit milk.

A Pheromone in Rabbit Milk?

At this point, 2MB2 could not be claimed to qualify as a pheromone: it was at best only a "candidate substance for pheromonal mediation" (Doty 2003). To assess the adequacy of entering it into the class of biologically active substances named 'pheromones', 2MB2 had to be further submitted to a set of functional tests. To that end, the initial Karlson and Lüscher's (1959) definition of the pheromone concept is of less help than subsequent definitions, which included more operational criteria (Beauchamp et al. 1976; Johnston 2000). These criteria imply that the candidate compound(s) is (are) (1) chemically simple (one or a very small set of compounds in a given ratio), (2) release(s) unambiguous, morphologically invariant, and functionally obvious behavioural responses in a receiver (3) in a highly selective and (4) species-specific manner; finally, (5) the coupling between the stimulus and the response should depend minimally or not at all on previous sensory experience.

Regarding criterion 1, 2MB2 being composed of a single molecular compound it is clear that it is the chemically "simplest" possible signal. Criterion 2 was addressed in comparing the form and frequency of responses triggered by pure 2MB2 and milk: the macroscopic structure of pup responses could not be differentiated between both stimuli, indicating that a key compound isolated from milk can elicit the same immediate response as the milk itself. Further, 2MB2 was highly efficient in triggering behavioural responses regardless of mode or context of presentation. Criterion 3, on the selective activity of the compound, was ascertained first by comparing the rate of pup responsiveness elicited by 40 odorants present or not in rabbit milk. These reference odorants were ineffective at any concentration (Coureaud et al. 2003). Thus, the behavioural effect of 2MB2 is highly selective and immune to novelty or non-specific arousal effects. Further, the activity of 2MB2 is limited within a range of concentrations extending over 5 log units (10^{-9} to 10^{-5} g/ml; Coureaud et al. 2004), so that there is perceptual leeway for signal fluctuations. Criterion 4 addressed the generality at the genus/species level of the coupling of 2MB2 perception with the typical searching-oral seizing behaviour. The generality of the releasing potency of 2MB2 was positively established in three ways: (i) pups from five different colonies of the same breed fed different diets were highly responsive to 2MB2; (ii) pups of eight different pure breeds or crossbreeds demonstrated very high reactivity (Schaal et al. 2003);

and (iii) the compound was highly active in wild-type pups (Kurz et al. 2005). Finally, the presentation of 2MB2 to newborn rats, mice, cats, and even closely related European hares (*Lepus europaeus*), came out with negative results (Schaal et al. 2003).

Finally, criterion 5, the trickiest to assess, relates to the fact that pup responsiveness to 2MB2 develops independently of previous exposure to 2MB2. In principle, early neonatal responsiveness to a given odorant can be ascribed to prenatal acquisition, to facilitated learning during the natal process, or to rapid learning immediately after birth (Schaal and Orgeur 1992). To rule out such postnatal or natal processes, the responses of pups were compared after (i) normal birth with exposure to the doe (warranting prenatal, natal and postnatal exposure), (ii) normal birth with separation from the doe (prenatal and natal exposure), and (iii) Caesarean delivery and postnatal isolation (prenatal exposure). Pups from these three groups responded at the same high rate to 2MB2, indicating that natal and/or postnatal processes do not differentially affect 2MB2 activity. Prenatal learning of 2MB2 was discarded by the fact that either amniotic fluid (collected at mid-and late pregnancy) or blood from pregnant does was ineffective (Schaal et al. 2003). Additionally, 2MB2 could not be detected by GC-MS in these substrates, suggesting that its activity does not derive from prenatal induction (as was noted above for the attractive potency of various food aromas). The above results allow qualifying 2MB2 from rabbit milk as a pheromone, both in the sense of the initial, loose definition of Karlson and Lüscher (1959), and in the sense of the later revisions proposed by Beauchamp et al. (1976) and Johnston (2000). As 2MB2 appears to be produced somewhere in the mammary tract (see below), and emitted in milk it was named "Mammary Pheromone" (MP).[6,7]

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[6] It is remarkable that a single compound can account for the activity of a highly complicated mixture of volatile and non-volatile compounds such as milk. This is in sharp contrast with most other putative mammalian pheromones, which tend to lose their releasing activity after separation from the original mixture (e.g., Beauchamp et al. 1976; Müller-Schwarze 1992).

[7] Several authors have suggested that when the impact of a pheromonal agent cannot be restricted to only one behavioural effect, it is sounder to relate the denomination of it to its source rather than to its effect(s) (Bronson 1971; Macintosh 1985). As the present pheromonal molecule 2-methylbut-2-enal bears multiple behavioural and cognitive consequences (releasing, at the least, immediate arousal, approach motions, head scanning movements, oral grasping, sucking, and engaging immediate associative learning), it was named "mammary pheromone" rather than the previously used "nipple-search pheromone". Although both wordings make use of the term 'pheromone', they cannot be held for equivalent. The former describes a chemically based pheromone and the systematic behavioural testing of it, whereas the latter denotes an extensively investigated behavioural phenomenon in which the pheromonal mediation is hypothetical. So far, no chemical analyses do permit to conclusively infer that the same compound(s) contribute(s) to the behavioural activity of rabbit milk (in which the mammary pheromone was identified) and of the nipple skin surface or the doe's ventral fur. Thus, *based on the available scientific evidence*, the identified signal was termed "mammary pheromone" not to endorse any misapprehension that both notions designate the same reality.

Some authors have insisted on the fact that the biological activity of pheromones should be viewed in the background of their adaptive value. Accordingly, to be qualifiedly named “pheromone” any given compound should not *only* fulfil a set of physical, chemical or biological criteria such as those reported above, but it should also be demonstrated that it functions as a signal in communicative exchanges which promote *mutually beneficial* consequences in the interacting conspecifics. So far, the functional involvement of the MP in the beneficial reciprocity of the rabbit doe and her litter can only be stated at a general level, at which it appears straightforward. On the offspring side, the MP has a key role in the immediate causation of a vigorous collective arousal response of the litter when the doe enters the nest, then in locating a nipple (and re-locating other nipples when switching between nipple during a same nursing bout), and finally in sustaining searching and sucking. Through its facilitation of milk intake, the MP obviously promotes survival and normal thriving until the transition to solid food. On the females’ side, the MP is one signal that releases probing and searching by the pups, and then that boosts tactile stimulations known (from comparative evidence) to trigger and sustain lactational physiology. Ultimately, the MP is related to the individual fitness of pups, and hence to the inclusive fitness of females.

It is agreed that such a beneficial reciprocity remains close to conjecture at this general level, but it opens a ground for future experiments. Special efforts should be devoted to investigate whether the MP and other common odour cues contribute to adaptive development in feeding and social cognition. For example, it could be shown that individual pups that do not react to the MP on postnatal day 1 have lower survival chances during the following 4 weeks (Coureaud et al. 2000d), indicating that initial responsiveness to the MP is linked with long-term viability. The mediating factors of such a phenomenon, possibly linked with perceptual or behavioural deficits in individual pups, are under scrutiny. Otherwise, during the first few days after birth, the oral grasping responses of pups to the MP appear to be compulsory at each presentation. They come under the progressive control of circadian or post-ingestive factors around day 5 (Montigny et al. 2004, 2006). This suggests that in the very first postnatal days, the MP supports a system of automatic response that may ensure constant readiness of pups to search and grasp nipples at any time when they are made available by the doe.

Another point of functional interest is the restricted period of effectiveness of the MP that closely matches the period when pups need to contact the nipples, viz. between birth and weaning. The activating effect of the MP on pups is strongest during the first 10 days, and then decreases when the other sensory systems become functional (Coureaud 2001; Kurz et al. 2005). On the female’s side, the MP content of milk decreases also notably over the 4-week lactation period, indicating a change in the pheromonal signal both on the receivers’ and the emitter’s sides (Coureaud et al. 2002b). Additional adaptive functions of the MP are described below.

Source(s) of the Mammary Pheromone

The emission of active odour cues by female rabbits is the greatest by the end of gestation and during lactation and tightly linked with the endocrine control of lactation (Hudson and Distel 1984; Gonzalez-Mariscal et al. 1994). As noted above, several studies have located the source of particularly active odour cues on the abdomen and mammary area of lactating does, the nipple surface, or in milk (Schley 1976; Müller 1978; Keil et al. 1990). This was also corroborated by tests consisting of putting pups in direct contact with (Hudson and Distel 1983) or above (without contact; Coureaud and Schaal 2000; Coureaud et al. 2001) the abdomen of lactating does.

Recent data confirm that the nipple itself is the source of the cues that elicit searching and grasping in newborn rabbits (Moncomble et al. 2003, 2005). The issue was approached by presenting pups with samples of skin tissues excised at various distances from the nipple or with milk collected from different levels of the mammary tract. The results indicate that active odorants are formed or distributed in two locations of the nipples in lactating females: "outer cues" are spread over the nipple epidermis, while "inner cues" are emitted in milk. The outer cues are restricted to the columnar part of the nipple and apparently do not extend over the adjacent furry skin surface. The source of inner cues appears to be restricted to the distal part of the galactophorous ducts, as mammary tissue samples taken under the nipple are behaviourally inert. The outer cues on the surface of the nipple and, to a lesser extent, the inner cues dissolved in ejected milk, have a similar behavioural potency than the pure MP (Moncomble et al. 2005). Both kinds of cues are thus *functionally* overlapping as suggested by similar responses of pups to the nipples of lactating females in vivo, to freshly excised nipples, to fresh milk or to the pure MP. It can be easily envisaged that if the source of the MP is in the distal part of the galactophorous ducts, it may contaminate the tip of the nipple and becomes locally entrapped in sebum and stratum corneum. Thus, a same source may well be responsible for the attractiveness of milk and of the nipple surface[8]. Chemical analyses are needed, however, to clarify this point.

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[8] This possibility may reconcile the behavioural effect of the 'nipple factor' (called "nipple-search pheromone", although no corresponding compound was formally identified) evidenced by the Giessen group (Schley 1976, 1977, 1979; Müller 1978) and extensively investigated by the Munich and Tlaxcala groups (Hudson and Distel 1983, 1984, 1995; Gonzalez-Mariscal and Rosenblatt 1996), and the 'mammary pheromone' identified as 2-methylbut-2-enal and which properties have been the object of systematic assessment according to an operational definition of the concept of pheromone (Beauchamp et al. 1976; Doty 2003).

The Mammary Pheromone in the Context of a Myriad of Common Odour Cues

Rabbit pups respond to multiple odour cues encountered in prior stages of development (section 2) and to one odour signal, the MP, which is highly active without prior exposure (section 3). Their behaviour seems thus driven by two types of chemosensory mechanisms, plastic mechanism(s), which are calibrated by circumstantial olfactory experience in preceding environments, and a predisposed mechanism seemingly hard-wired for the detection of a given compound. Do these postulated mechanisms interact? And if it is the case, which forms might such interactions take? First, both mechanisms may be underlain by the same system[9]; accordingly, an odour newly learned while nursing would easily replace the inborn effect carried by the MP, presupposing that the pheromonal activity is labile and easily re-assignable to common odour cues. Second, predisposed and plastic mechanisms may be completely independent even though they both eventually recruit the same motor effectors. Thus, learning a novel odorant in conjunction with suckling should be without impact on the functional responses elicited by the MP. Third, the predisposed mechanism may control the operations of the plastic mechanisms; in other words, when the MP is made contingent with any other common odorant, this latter stimulus is automatically assigned a special psychobiological significance.

Some of these interactions between the pheromone and common odour cues have been examined. The strong response of rabbit pups to the pure MP remains unaffected by deprivation of exposure to it during the first 6 days after birth (Coureaud et al. 2000c). Otherwise, when MP-deprived pups learn an artificial odorant in association with bottle-feeding, this odour becomes as active as the MP in releasing typical responses. However, although never previously exposed to the MP, these pups remain highly reactive to it despite the fact that the newly learned odour can also elicit oro-motor responses (Coureaud et al. 2000c). In sum, pup responsiveness to the MP is neither labile nor easily replaced by newly learned odours, suggesting functional dissociation of the predisposed pheromonal mechanism and of plastic common olfactory mechanisms. This dissociation does appear to be asymmetrical, however. The

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[9] A simplistic functional division of nasal chemoreception would assume that the MP is processed through the vomeronasal pathway, while common odour cues are processed through the main olfactory pathway. The current, incomplete, evidence suggests, however, that both predisposed and plastic postulated systems are located in the main olfactory system and not in the accessory olfactory system. This is provisionally supported by the facts that the stimulation of pups with the pure MP does not at all activate the accessory olfactory bulb (as assessed by local 2-deoxyglucoce intake; Saucier et al. 2004), and that the lesion of the vomeronasal pathway does not hinder the expression of searching-grasping response in pups put on a lactating does' abdomen (potentially giving off the MP as well as other odour cues; Hudson and Distel 1986).

predisposed mechanism has a powerful influence on plastic olfactory mechanisms, following the third hypothesis above. Any odorant made contingent with the MP incorporates the behaviour-releasing properties of the MP. This transfer of reactivity occurs after only one simultaneous exposure to the odorant and the pure MP, and it does not strongly depend on the state of arousal of the newborn pup or on the expression of sucking (Coureaud et al. 2005). In addition, learning an odour in the absence of the MP is slow, because it can take up to six associative episodes (by bottle-feeding) for a common odorant to attain the response rate of the MP (Coureaud et al. 2000c). Thus, although both predisposed and plastic olfactory mechanisms are experimentally dissociable, they are linked in the nursing situation, making possible their participation in the rapid amplification of the range of odorants that govern early behaviour.

These results demonstrate once more that the MP released by nursing rabbit females canalises the pups' adaptive response to the challenge of getting milk under extremely constrained conditions: it triggers immediate arousal, sets pups in directional motion, evokes rapid rooting movements ended by oral grasping of a nipple, and it eventually boosts the rapid acquisition of unanticipated common odour cues which may be diluted in milk or adhere to the mother's abdominal surface (Coureaud et al. 2006) In sum, the predisposed olfactory mechanism based on the MP can be seen, in addition to its obvious releasing effect on behaviour, as an extra-fast magnifier of the cognitive capabilities of rabbit pups.

Conclusions

The evidence provided in the present chapter illustrates how rabbit newborns, pre-weanlings, and weanlings are accomplished organisms at each stage of development, and how early events determine strategies to cope with the transitions leading to the feeding and social ways of adult *Oryctolagus*. The interplay of behaviour, learning, and physiology are central in this ontogenetic progression. In fact, learning processes around the nipples and milk are overdetermined in newborn rabbits, as they are in other newborn mammals (e.g., Alberts 1987; Schaal 2005), involving at the same time passive, mere exposure effects, as well as associative mechanisms relying on a variety of reinforcers. Among such reinforcers, *Oryctolagus* females have evolved a way to canalise the behaviour in their offspring: they emit a mammary pheromone that instantly releases activation and stimulates directional searching in pups. This pheromone further accelerates the learning of common odorants present on the female's belly, specifying them as cues that predict nipples and milk. Through this pheromone, female rabbits have evolved an efficient means of enabling their offspring to rapidly expand the repertoire of odour cues. Future investigations should be directed to the role of MP-induced odour learning in the adaptive responses of growing rabbits to feeding transitions (initiation of caecotrophy, weaning, adult food flexibility) and social integration (maternal and kin recognition, mate selection and preference).

Rabbit females may have another essential role: they set on the digestive physiology of their offspring. Right after birth, they place faecal pellets in their pups' immediate vicinity. Pups are then *olfactorily* driven to these pellets, nibble, and eat them (Moncomble 2006). This observation points to the possibility of an additional chemosensory trigger afforded by lactating females to initiate another vital end-point: inception of caecotrophy and microfloral colonisation of the gut (Moncomble 2006).

As it seems improbable for any species to base offspring survival on a single mother–young signalling strategy, the possibility that multiple chemosensory signals promote viability and fitness should be investigated in the rabbit, and more broadly, in Lagomorphs. This mammalian order would provide excellent conditions for comparative and phylogenetic investigations on the sensory balance that controls female–offspring communication, and specifically on whether specialised chemosensory signals (such as the mammary pheromone of the European rabbit) are the ultimate consequence of life history patterns requiring females to adopt an offspring protection strategy by staying away from them.

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Mother-Young and Within-Litter Relations in the European Rabbit *Oryctolagus cuniculus*

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Introduction

The European rabbit, *Oryctolagus cuniculus*, in its domesticated form, is an important animal in biomedical research and in a number of countries is of economic significance for the production of meat and fur. In the wild, it is an appreciated game species, and in several countries it is also a major agricultural pest. The rabbit's long association with humans has resulted in a large body of information on its general biology and it is now one of the most studied mammals in the wild. In whatever context, however, the rabbit is best known for its remarkable reproductive capacity. This is due in large part to the reproductive efficiency of the female (Fig. 1), a notable feature of which is the brief time mothers spend each day even with their newborn young (reviewed in Hudson and Distel 1982; 1989). After giving birth to the altricial young in a separate nursery burrow or in a chamber in the colony warren, the doe leaves, closes the entrance, and only returns to nurse for a few minutes once approximately every 24 h. As for several other lagomorphs (see below), such limited contact between mother and young is thought to have evolved to reduce the possibility of predators locating the nest from the attendance of the more conspicuous mother (Zarrow et al. 1965).

Rabbits have a postpartum oestrus and usually mate again soon after giving birth. If this results in a further pregnancy, the doe abruptly weans the young she is currently nursing on about postnatal day 26 in preparation for the birth of the next litter (reviewed in Hudson et al. 1995a; 1996; Martínez-Gómez et al. 2004).

This, for a mammal, unusually limited maternal care is made possible by a number of behavioural and physiological specialisations of mothers and young. It also makes it possible to study interactions among littermates and to explore the consequences of these for development, survival, and later reproductive success, without the complicating factor of the mother's presence. In this chapter we review current knowledge of the mother–young relationship,

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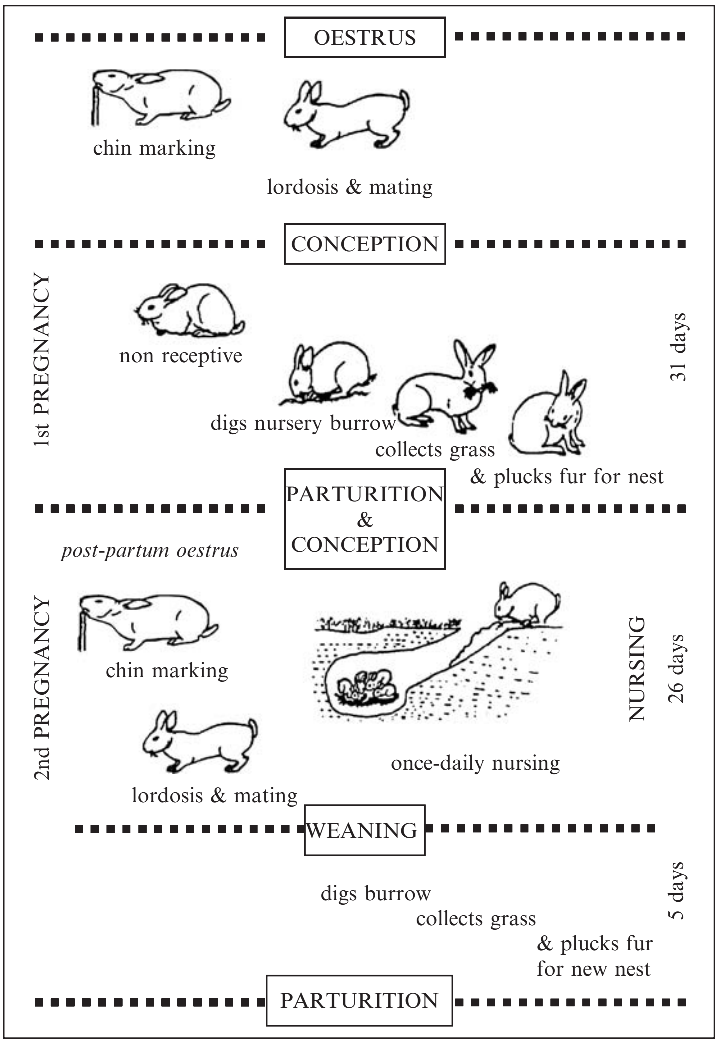

Fig. 1 Reproductive cycle of the female rabbit illustrating the stereotyped pattern of maternal behaviour and overlapping production of litters

together with our recent studies of interactions among littermates and the potential contribution of such information to understanding individual life histories. Although we only consider the European rabbit, given the similarly limited maternal care shown by other lagomorphs (*Silvilagus floridanus* Ecke 1955; *S. aquaticus* Sorensen et al. 1972; *Lepus europaeus* Broekhuizen and Maarskamp 1980; Broekhuizen and Mulder 1983; Broekhuizen et al. 1986; *Lepus timidus* Dyballa 1978; *L. americanus* Rongstad and Tester 1971) (reviewed in Cowan and Bell 1986), the patterns and questions outlined below should apply broadly to them also.

Mother–Young Relations

Maternal behaviour in the rabbit begins with the mother digging a nursery burrow or a chamber in the colony warren late in gestation. She constructs a nest in this, sometimes only hours before giving birth, of dry grass and fur plucked from her chest and flanks (Fig. 1). Nest-building and its hormonal control have been well reviewed by González-Mariscal and Rosenblatt (1996).

Birth

Like other nocturnal mammals, rabbits typically give birth during the daytime, that is, during their rest period (Hudson et al. 1995a; Ninomiya-Alarcón et al. 2004; reviewed in Jilge and Hudson 2001). Birth is rapid and is usually accomplished within 10–15 min even for the large litters of ten or more young common in some domestic breeds (Fuchs and Dawood 1980; Hudson et al. 1999a). This is important since the long vagina of the rabbit (Martínez-Gómez et al. 1997) results in rupture of the umbilical cord and suffocation of the pups if they are not rapidly expelled. During parturition, the mother stands hunched over the nest, sometimes briefly licking the pups as they are born and rapidly eating the placentas. The pups are usually very active, rapidly free themselves from the amniotic membranes, and may even suckle while parturition is still in progress. After the birth of the last pup, the doe leaves and only returns to nurse briefly once approximately every 24 h (see below) (Hudson et al. 1999a; reviewed in Hudson and Distel 1989; Jilge and Hudson 2001).

As in other mammals, the physiological mechanisms triggering parturition at an appropriate time of the 24-h day are still not completely understood (Fuchs and Dawood 1980; Hudson et al. 1995a; Ninomiya-Alarcón et al. 2004).

Circadian Synchrony

Both in the laboratory and wild, does return each night to the nest to nurse for just 3–4 min (reviewed in Hudson and Distel 1989; Jilge and Hudson 2001). The pups anticipate and prepare for this vital event behaviourally and physiologically (Hudson and Distel 1982; Jilge 1993, 1995; Allingham et al. 1998; Jilge et al. 2000, 2001; Caldelas et al. 2005; reviewed in Hudson and Distel 1989; Hudson 1998; Jilge and Hudson 2001). An hour or so before the doe's arrival they become increasingly active, increasingly responsive to vibrational and tactile stimuli, and gradually uncover from the nest material. This enables them to reach the doe's ventrum unhindered and to start the rapid search for nipples the moment she stands over them (see below). This anticipatory arousal is accompanied by a rise in the pups' (and mothers') body temperature (Jilge et al. 2000; 2001), and in fasted pups, in elevated serum concentrations of free fatty acids (Escobar et al. 2000), presumably in preparation for the competitive nursing situation soon to follow (see below). This state of arousal may also explain the interesting finding that newborn pups briefly exposed to the smell of a human hand around nursing time show less fear of humans post weaning (Pongrácz and Altbäcker 1999, 2003). During or immediately after nursing, the pups urinate copiously, dry off as they burrow back under the nest material, and then regroup to form a huddle in the thermally most advantageous part of the nest (Hudson and Distel 1982; cf. Pacheco-Cobos et al. 2003).

Anticipation of nursing represents an endogenous circadian rhythm and does not simply depend on emptying of the gut. Thus, litters that miss a feed show an increase in activity and body temperature in anticipation of the next nursing 48 h later (Hudson and Distel 1982; Jilge 1993, 1995; Jilge et al. 2000, 2001; reviewed in Jilge and Hudson 2001).

Little is known as to how this rhythmicity is achieved. However, the fact that rabbit pups can survive at least 2 days without suckling and that the expression of immediate early and "clock" genes has been demonstrated in the brain of pups in association with suckling provides a promising basis for future research (Allingham et al. 1998; Caba et al. 2003; Caldelas et al. 2005).

Nursing and Suckling

Having prepared for the mother's arrival, the pups must find the nipples, attach, suck and obtain sufficient milk to survive for at least the next 24 h. They do this without direct behavioural assistance from the doe. When the doe enters the nest she simply stands over the litter, remaining virtually motionless until, after 3–4 min, she deposits a few hard faecal pellets, abruptly jumps away and leaves (Hudson and Distel 1982; Hudson et al. 1996; Bautista et al. 2005). Although she might briefly lick the pups, she does not interact with them in any other obvious way. Even if she stands on a pup she will not necessarily adjust her posture to release it (Hudson and Distel 1982). When she jumps away from the litter at the end of nursing, the pups drop immediately from the nipples. This prevents them being dragged from the nest, which is important since rabbits do not retrieve their young (Ross et al. 1959; own observations).

Immediately after the mother adopts the nursing posture, the pups rear up and push their muzzles deep into her ventral fur to start the rapid, stereotyped search for nipples. Using tactile and pheromonal cues, they locate and attach to nipples within seconds (Hudson and Distel 1983; Distel and Hudson 1985; Bautista et al. 2005). Emission of the so-called nipple-search pheromone, essential for locating the nipples and suckling, depends on females' reproductive state; short photoperiod or anoestrous is accompanied by little or no emission, long photoperiod or oestrus by moderate levels, and late pregnancy and lactation by high levels (Hudson and Distel 1984, 1990). Ovariectomised females show virtually no emission, but increasingly greater levels when treated with a sequence of estradiol, plus progesterone, plus prolactin (Hudson and Distel 1984; Hudson et al. 1990; González-Mariscal et al. 1994; reviewed in Hudson and Distel 1995b).

Chemical cues releasing nipple-search behaviour and nipple grasping are present in the milk (Schley 1981; Keil et al. 1990), and a compound in the milk releasing grasping, 2-methylbut-2-enal, has been recently identified (Schaal et al. 2003; Moncomble et al. 2005). However, it is still not clear if a single substance can account for the complete nipple-search sequence, nor how the cues come to be distributed on the doe's ventrum in such a way as to release the search behaviour and guide pups to nipples, and then even in non-lactating does (Hudson and Distel 1983, 1990; Distel and Hudson 1984; Hudson et al. 1990). It is also unclear whether the pups' response to the pheromonal cues governing suckling is learned or is genetically determined. Pups delivered by Caesarean section, and thus without postnatal experience, show normal nipple-search behaviour (Hudson 1985). However, reports that they can learn odours prenatally (Bilkó et al. 1994; Altbäcker et al. 1995; Semke et al. 1995) make it difficult to exclude learning, and particularly as odours encountered prenatally enhance suckling performance when encountered later on the mother's ventrum (Coureaud et al. 2002). Indeed, pre- and postnatal transmission of olfactory information provide a potential means for female rabbits to enhance the survival of their young in a variety of contexts (Bilkó et al. 1994; Altbäcker et al. 1995; Coureaud et al. 2002; reviewed in Hudson et al. 1999b).

Weaning

The transition to solid food begins already in the nest. Around day 12, the pups start to nibble the hard faecal pellets deposited by the doe, and around day 14 they start to eat the nest material (Hudson et al. 1996). Since the quantities eaten are small, this probably helps prepare the young for the digestion of solid food, for example, by providing appropriate gut flora rather than being of direct nutritional benefit. This, however, remains to be investigated.

Milk production starts to decline around day 20 (Lincoln 1974; Hudson et al. 1996), and if the doe is pregnant from postpartum mating, she will stop nursing the current litter around day 26 (Lincoln 1974; Hudson et al. 1996). Whereas the previous day she shows normal nursing behaviour, 24 h later she refuses to nurse and under confined laboratory conditions may even attack the young attempting to suckle. With the arrival of the next litter several days later the usual once-daily nursing rhythm is resumed.

Abrupt weaning by pregnant does (non-pregnant mothers nurse longer) probably serves to prevent the sudden release of oxytocin accompanying nursing from inducing the premature birth of the next litter (Lincoln 1974; Fuchs et al. 1984; Hudson et al. 1995a, 1996; Ninomiya-Alarcón et al. 2004). However, the neuroendocrine mechanisms regulating this precise go-stop-go of nursing are unknown.

Within-Litter Relations

Most mammals grow up in the company of the same or different age sibs (or half sibs) and relations among them can be expected to affect individual development, survival, and fitness (Mock and Parker 1997; Sulloway 2001). The newborn rabbit provides a particularly good opportunity to investigate this given that its social life is defined almost exclusively by its littermates. The rabbit's system of absentee mothering also means that the influence of siblings on individual development can be studied to a degree not possible in most other mammals. Being born into a nest of littermates confronts a pup with an interrelated array of challenges and developmental possibilities. These can be broadly grouped according to two functional contexts, suckling and position within the litter huddle; the first relating to the need to obtain sufficient milk for survival and growth, and the second to the need to maintain an adequate body temperature and to obtain somatosensory stimulation and experience of conspecifics necessary for normal sensorymotor and social development.

Suckling

Although rabbits usually have, depending on the breed, between six and eight nipples (Patton 1994; own observations), competition among littermates for milk is severe. A high percentage of pups fail to obtain milk during at least one nursing event and up to 20% die of starvation within the first postnatal week even under the relatively favourable conditions of the laboratory (Coureaud et al. 2000; Drummond et al. 2000). Competition is particularly severe given that pups only obtain significant amounts of milk during the second minute of nursing (Fig. 2; Bautista et al. 2005).

Viewing pups' behaviour during nursing in a glass-bottomed nest box showed that they compete for nipples in a vigorous scramble and without obvious signs of overt aggression. A preference for particular nipples or a "teat order" was not seen and nipple pairs appeared to be equally productive. Body mass is a good predictor of suckling success and, independent of sex, the heaviest pups at birth obtain more milk, are more likely to survive and are heavier at weaning than their lighter sibs (Fig. 2; Drummond et al. 2000; Bautista et al. 2005).

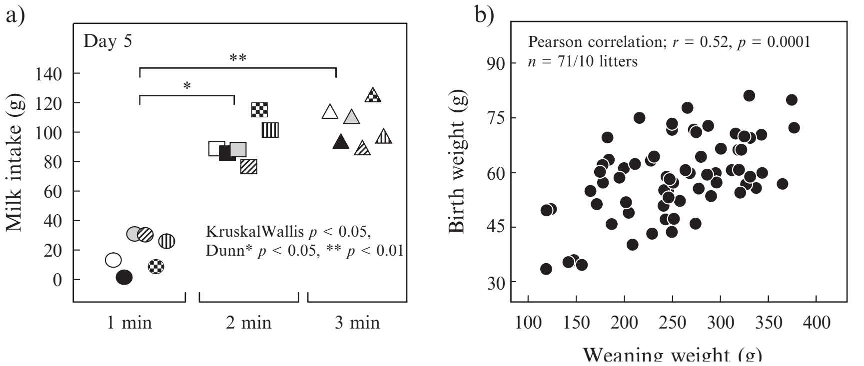

Fig. 2 a) Milk intake by litters of domestic rabbits on postnatal day 5 when nursing was interrupted 1, 2, or 3 min after the doe assumed the nursing posture (each symbol represents a different litter; six pups/litter). Pups obtained almost all milk during the second minute (adapted from Bautista et al. 2005). b) Positive correlation between birth weight and weaning weight in domestic rabbits (adapted from Drummond et al. 2000)

Despite such competition, sibling presence is also of benefit during nursing. Does do not receive sufficient stimulation of the nipples to elicit the quiet nursing posture necessary for successful suckling (own observations) or to stimulate the release of oxytocin necessary for milk letdown if they are only given a single pup to nurse (Fuchs et al. 1984). The costs and benefits of sibling presence for individual littermates, including in relation to thermoregulation as outlined below, raise the question as to whether there is an optimal litter size. While preliminary analysis in our laboratory suggests that a litter size of around six might be optimal for the chinchilla breed we work with, any such estimate is likely to vary among breeds, as well as between domestic and European rabbits.

The Litter Huddle

Between suckling episodes, pups also compete for well-insulated, central positions within the litter huddle and expend considerable energy climbing over and burrowing under each other in a continuous effort to achieve and maintain these positions. Again, body mass is a good predictor of the outcome of such struggles, with heavier pups of either sex generally having a higher huddle index (= % of an individual's periphery in contact with another pup + % of its dorsal body surface covered by another pup × 2, + % of its ventral body surface covering another pup) than their lighter sibs (Fig. 3; Bautista 2005). Moreover, pups with a high huddle index also have a higher index of converting milk to body mass (= increase in pre-nursing body weight during $t + 1$ days/weight of milk ingested over t days; Bautista et al. 2003; 2005).

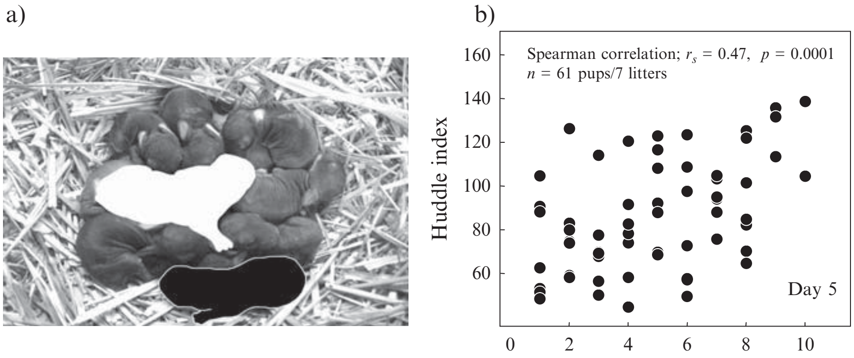

Fig. 3 a) Huddle of 3-day-old domestic rabbits showing a large pup (*white*) in a central position with considerable body contact with littermates and a smaller pup (*black*) in a peripheral position with less body contact. b) Positive correlation on postnatal day 5 between individual pups' within-litter rank in body weight and their huddle index, meaning the degree to which their body surface was in contact with littermates (see text for explanation; adapted from Bautista 2005). For each rank, each point represents a pup from a different litter (n = 7 litters of 7 to 10 pups) so as to avoid pseudoreplication

Aside from such competition, for any individual, littermates represent a substantial thermoregulatory resource. Thus, pups raised together, even with a single littermate, have higher mean body temperatures and a higher index of converting milk into body mass than a randomly selected littermate reared under the same conditions but alone (Fig.4; Bautista et al. 2003).

Early sibling presence might also provide pups with somatosensory stimulation enabling them to anticipate and prepare for the daily nursing visit and enhancing their motor development. In support of this, we found that five pups raised together obtained more milk during the daily nursing and had better postural control when tested daily on a ramp inclined at 15° than a randomly chosen sixth littermate raised alone (Fig. 5). Early littermate presence might also contribute to longer-term social competence, since we found that five pups raised together competed more successfully for water and food post-weaning when they were paired with a sixth isolation-raised littermate of similar weight (Fig. 6).

From these first findings we conclude that the European rabbit provides a particularly good opportunity for investigating the contribution of the early social environment to the emergence of individual behavioural phenotypes. Questions of future interest include the influence of early sibling interactions on individual long-term survival and reproductive success, on the development of successful alternative strategies in achieving these goals, identification of physiological mechanisms accompanying such differences, and the extent to which findings in the laboratory correspond to reports under natural conditions in the wild.

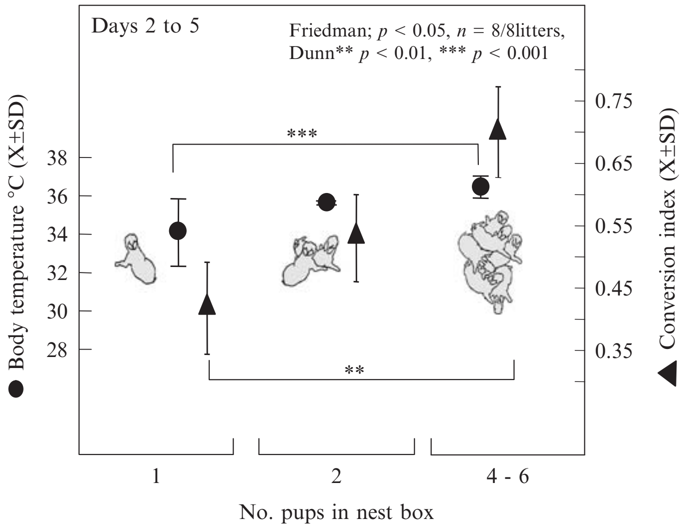

Fig. 4 Pups kept together from postnatal days 2 to 5 and able to form a huddle had higher mean body temperatures and a higher index of converting milk to body mass (see text for explanation) than their littermates kept under identical conditions but with only one other sibling or with none (adapted from Bautista et al. 2003). For each condition, data from only one (randomly selected) pup per litter was used so as to avoid pseudoreplication

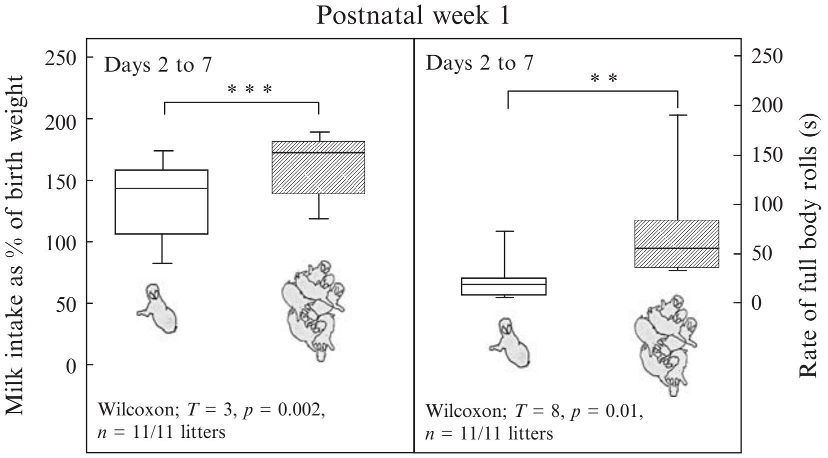

Fig. 5 Pups kept together with five littermates obtained significantly more milk (*left panel*) and had a lower rate of overbalancing when tested on a ramp (*right panel*) on postnatal days 2 to 7 than a randomly chosen sixth littermate raised alone. For each condition, data from only one (randomly selected) pup per litter was used so as to avoid pseudoreplication. *Dark horizontal bars* inside the boxes are medians, *horizontal limits* of the boxes = 25th and 75th percentiles, *error bars* = 10th and 90th percentiles

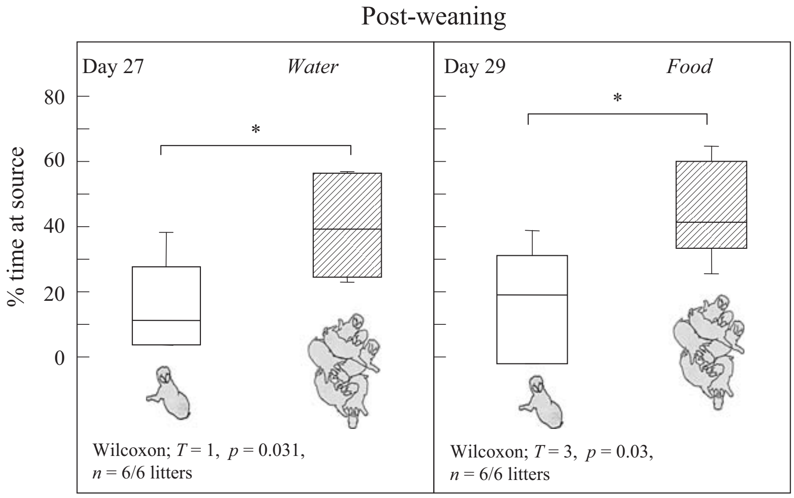

Fig. 6 Pups reared together with five littermates competed more successfully post weaning for water (*left panel*) and for food (*right panel*) when paired with a littermate that had been raised alone (see text for explanation). Animals were water or food deprived 24 h before testing; explanation of plots is the same as for Fig. 5

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Fertility and Infertility in the European Hare *Lepus europaeus* in Australia

PHILIP STOTT*,[1], STEPHEN HARRIS[2], AND NICOLE WIGHT[1]

Introduction

There are two lagomorph species in Australia, both of which were introduced ca. 1860 by European settlers (Rolls 1969). The European hare *Lepus europaeus* occupies a range of ca. 700,000 km^2 in the south-east (Jarman 1986), a distribution encompassed within the distribution of the European rabbit (*Oryctolagus cuniculus*) which now occupies an area of ca. 4,500,000 km^2, representing the southern two-thirds of the continent (Myers et al. 1989). Australian hare populations are presently at low densities due to unknown factors, despite a history of plague densities and considerable environmental and economic losses in some regions (Douglas 1972).

There is much published information on the physiology and ecology of European rabbits in Australia, whereas the hare has been neglected as a focus for study (see Jarman 1986). This study investigated the reproductive output by season of the European hare in Australia, and also investigated the prevalence and expression of infertility in adult female hares to ascertain whether disease was influencing the reproductive output of Australian hare populations.

Materials and Methods

Hunters submitted the carcasses of female hares shot on the Volcanic Plains (VP) of western Victoria (centred at 37°50'S, 142°04'E), the Monarto Plains (MP) of South Australia (35°14'S, 139°05'E), and the Chowilla Floodplain (CF) of South Australia (33°59'S, 140°55'E) (see Fig. 1), in each month of the year but spread over the years 1996–1999. VP and MP are both agricultural regions with pastures comprising grasses mixed with legumes such as medic and clover, but MP also has extensive areas of leguminous crops such as lupins,

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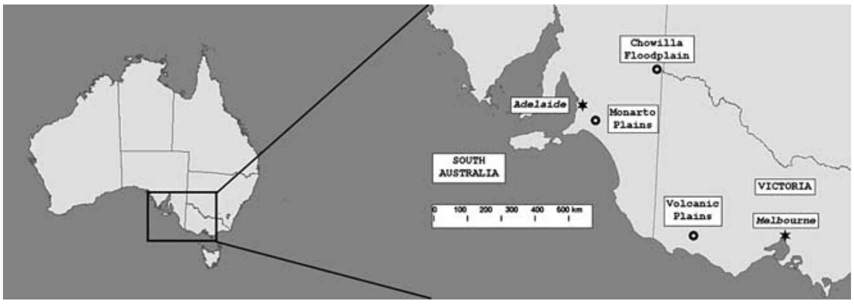

Fig. 1 Portions of the Australian states of South Australia and Victoria, showing the source sites of hares used in dissections. ○ - sites; ✱ - major cities; lines – state borders. Source of base map: Auslig (Australian National Mapping Agency)

soy bean, and vetch; whereas CF is a region of natural vegetation, lacking crops and pastures.

Carcasses were dissected within 6 h of death. Gross body weight was taken, the abdominal cavity was opened, and the mammary glands were examined for signs of lactation. The reproductive tract was removed and the fallopian tubes were inspected visually before the ovaries were separated and preserved in formalin for later examination, which consisted of visual inspection of serial sections taken at 1.0-mm intervals. The uterine horns were opened while fresh, and visually inspected for viable or resorbing foetuses, placental scars, or reproductive abnormalities. Placental scars (Hansen 1992) were counted and grouped by size and visual appearance. Selected tissue samples were preserved for later histopathological examination. Only undamaged uteri were used for analysis of pregnancy rate and litter size. Foetuses were separated from their placentas, blot dried, weighed to 0.1 g, and measured from crown to rump (CR, mm). The stages of gestation were ascertained from Broekhuizen and Martinet (1979) and projected to determine the dates of conception and birth.

There was no attempt to recover preimplantation blastocysts; in European rabbits, pregnancies are visible from day 4 of a 30-day gestation (Brambell 1942), so ca. 10% of pregnancies in the rabbit would be missed in a study relying on visual detection of pregnancy. For the same reason, pregnancies must also have been missed in our study. Gestation in the hare is of *c.* 45 days duration (Stavy and Terkel 1992) and birth occurs at a more precocial stage of development, but the stage at which visible uterine swelling occurs is not known; presumably, 7–10% of pregnancies would have been missed in my study of the hare.

For aging of hares, the eyes were removed and subjected to the dried eyelens method of Suchentrunk et al. (1991), and on that basis were divided into five age classes: leveret, juvenile, near adult, young adult, and older adult (defined in Table 1). For the analysis of reproductive abnormalities, age

Table 1 Age distribution of female hares from all sites as determined using dried eyelens weight

Age category	(No lens)	Leveret	Juvenile	Near adult	Young adult	Older adult	Total
Number	55	3	35	64	53	97	307

Table 2 Definitions of age classes used for hares

Age class	Leveret	Juvenile	Near adult	Young adult	Older adult
Eyelens (mg)	<102	102–185	185–245	245–297	>297
Age (days)	<31	31–122	122–234	234–365	>365

classes of older adults (defined as in Table 2) and younger adults (combining the near adults and young adults) were used.

The data for pregnancy and lactation rates for near adult, young adult, and older adult female hares were grouped into months. Corrections for sample size by month were applied. Conception rates by month and birth rates by month were derived from the stage of pregnancy. Recruitment by month was taken as the birth rate multiplied by the litter size, and the mean annual production of young per adult female was calculated according to the formula of Broekhuizen and Maaskamp (1981). The age of attainment of puberty was taken as the age at first conception.

When it became apparent that pathological changes were evident in the reproductive tracts of the adult female hares, Wight (1998) obtained samples of adult female rabbits (does) from the same regions and in most cases the same properties, and processed them in the same manner as for the hares but using the equivalent eyelens aging technique for rabbits (Myers and Gilbert 1968). Does less than 6 months of age or for which no eyelens was available were excluded from further consideration.

Differences between age classes of hares in mean litter size by month were compared by ANOVA with a Tukey post hoc test using the SPSS statistical program (SPSS Incorporated, Chicago). Means are presented as ± one standard deviation (SD).

The data for abnormalities were analysed using log-linear modelling assuming a Poisson distribution and a log link. The model includes the main effects, two-way interactions and the three-way interaction between Population (Volcanic Plains, Monarto Plains, and Chowilla Floodplain), Age (Younger Adult Female, and Older Adult Female), and Abnormalities (Present or Absent). The three-way interaction was assumed not to be significant and was used as the residual term in order to test the two-way interactions and main effects. The model therefore tested for a relationship between abnormalities and the explanatory factors of population and age. The data were then summarised in a 2×2 contingency table for abnormalities and age (i.e. collapsing over population) and a 2 × 3 contingency table for abnormalities and population (i.e. collapsing over age), and subjected to χ^2 tests for independence.

Results

Population Sampling

The total number of hare carcases available for dissection was 307. The age distribution is given in Table 1. Pregnant females were found in all months of the year. However, fertility varied seasonally, with a sudden rise in conceptions in late winter declining to a nadir in autumn (Fig. 2).

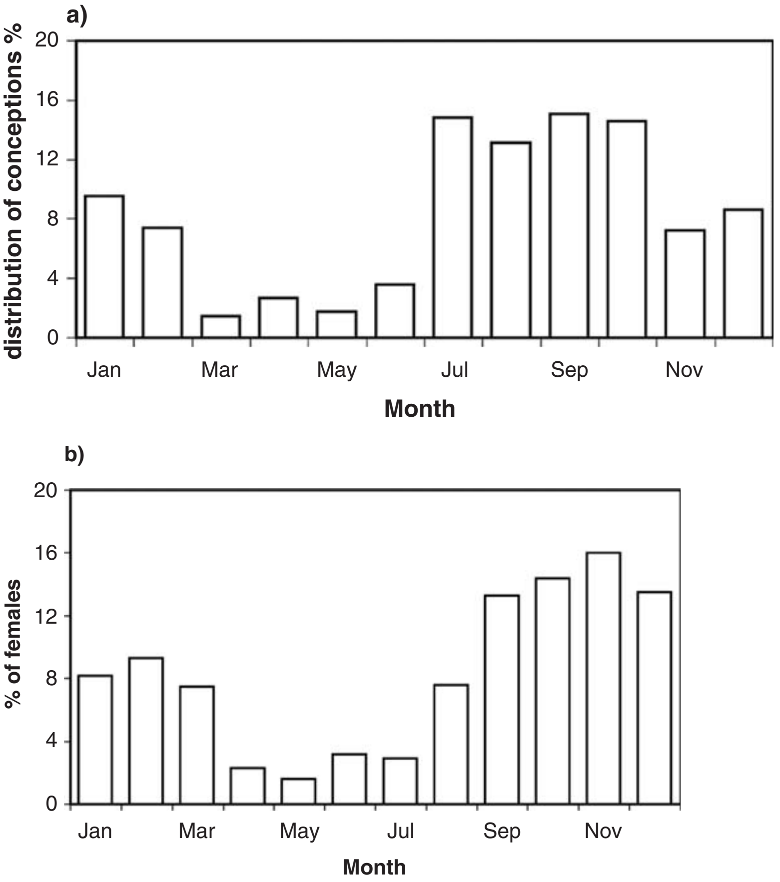

Fig. 2 **a)** Proportion of adult females conceiving by month; **b)** Proportion of adult females giving birth by month; continue next page

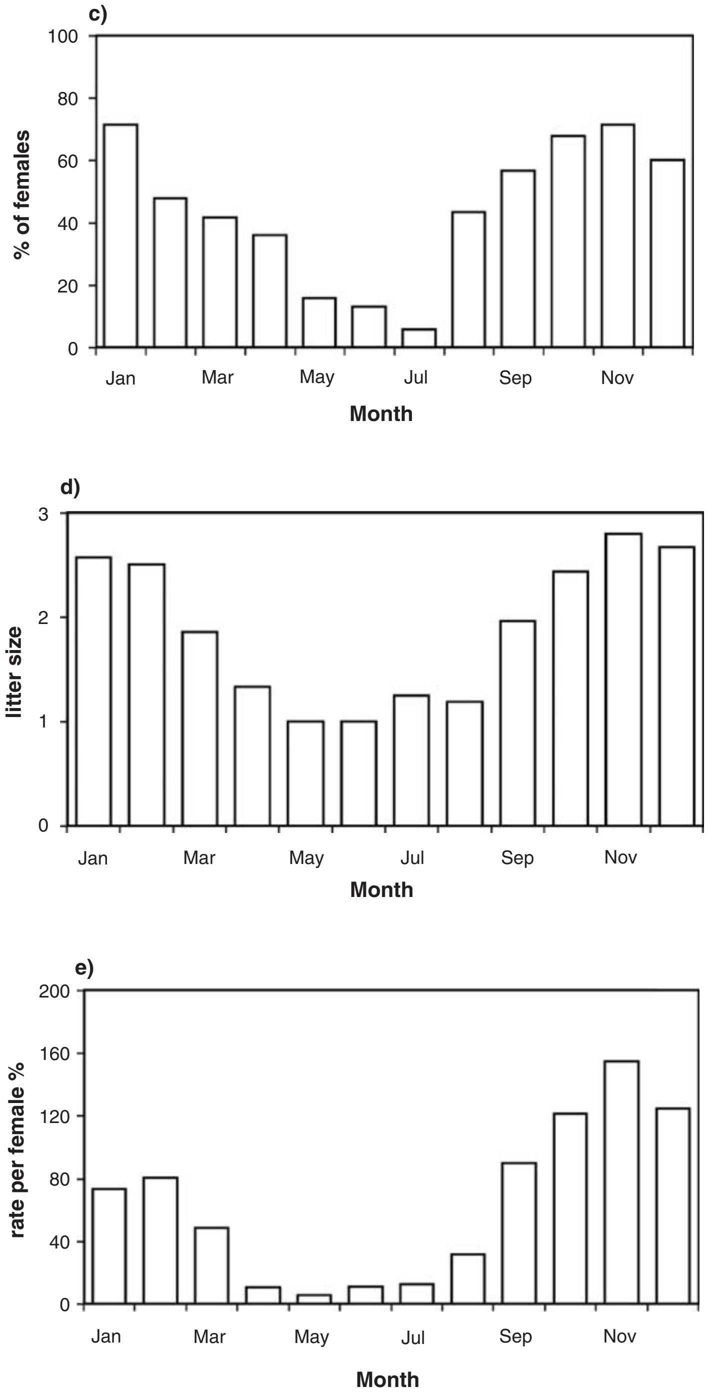

Fig. 2 (*Continued*) **c)** Proportion of adult females lactating by month; **d)** Mean litter size of adult females by month; **e)** Recruitment per adult female by month

Puberty

There were two pregnant juveniles, with the earliest conception occurring at 96 days of age if aged according to Suchentrunk et al. (1991) or 99 days if aged according to Bray (1998). The presence of placental scars indicated that another five animals had become pregnant as juveniles. Thirty pregnant animals had become pregnant as near adults, and the presence of placental scars indicated that another 16 animals had become pregnant as near adults. Overall, 9.2% of the female hares had become pregnant as juveniles, and 60.5% as near adults.

Reproductive Output

The first litter of the season almost invariably consisted of one leveret, but the number increased to a peak of 2.8 in November and was maintained near this level through summer before declining to return to one by late autumn (Fig. 2d). Mean litter size for the three populations was 2.19. The largest litter was carried by the largest hare (as assessed by paunched weight) and contained five leverets.

For adult female hares for which both pregnancy and placental scar data were available, the pregnancy rate declined with age (Table 3). Litter size was significantly different between age classes (ANOVA: $F_{0.05,2.95} = 6.90$), with near adult hares being significantly less fecund than the older age classes. The larger litter size in the older female population compensated to some extent for their lower fertility. The decline in fertility was associated with reproductive abnormalities evident in the older hares.

Reproductive Abnormalities

Reproductive abnormalities were found in 20.0% (51/245) of the hares examined, and were distributed as follows. Thirty-three hares exhibited macroscopically visible pathological changes in the uterus, which were diagnosed as

Table 3 Reproductive parameters of female hares by age group

Age class	Near adult	Young adult	Older adult
Pregnancy rate in female population	0.55 *n* = 58	0.54 *n* = 50	0.45 *n* = 82
Placental scar rate in female population	0.34 *n* = 58	0.60 *n* = 50	0.41 *n* = 82
Litter size in pregnant females	1.78 *n* = 32	2.37 *n* = 27	2.54 *n* = 39

cystic endometrial hyperplasia (25 cases), pseudopregnancy (2), endometritis (2), focal endometrial venous congestion (2), and myometrial thickening and fibrosis (2). Twelve of these 33 (but not including the pseudopregnant animals), together with another four animals, exhibited pathological changes in the fallopian tube(s). The macroscopic and histopathological appearances of the lesions are described in Stott and Wight (2004). None of these 37 hares was pregnant, and they were judged to be at least temporarily infertile.

The ovaries were available for 32 of the 37 hares: 20 contained corpora lutea (including both cases of pseudopregnancy), two had corpora haemorrhagica, six had follicles, and four showed no visible sign of activity. Lesions were present in 28.9% of all female hares known to be >12 months of age, but in only 2.8% of those known to be 4–12 months. Infertility in the older hares was recorded in all months, and was 46.2% at MP and 26.3% at VP, but none of the 19 older females at CF was infertile. The combined data for hares from MP and VP is presented as Fig. 3.

Reproductive abnormalities not necessarily related to fertility were also observed in some hares, and make up the balance of the abnormalities listed in Table 4. One hare had two completely macerated foetuses in one horn, and a single apparently normal foetus at 38 days gestation in the other, cysts were observed in the ovary of two animals, tumours occurred in the uterus and/or broad ligament in three animals and the mammary gland of another animal, four hares had adhesions involving reproductive organs, and two had extra-uterine mummified foetuses contained within intact foetal membranes. One of the hares with extra-uterine foetuses also had bilateral salpingitis, the hare with a mammary tumour also had cystic endometrial hyperplasia, and a hare with cystic ovaries also had a severe case of cystic endometrial hyperplasia involving the myometrium, hydrosalpinx, and adhesions between the uterus, parietal peritoneum, and intestines. Resorbing foetuses were recorded in 11 uteri, in six

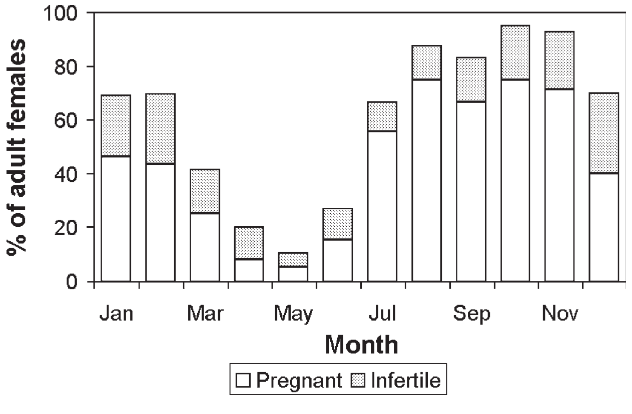

Fig. 3 Combined prevalence of visible pregnancy and infertility in adult female hares from the Monarto and Volcanic Plains by month

Table 4 Numbers of lagomorphs in each category by site. Jills – adult female hares. Does – adult female rabbits

	Chowilla floodplain		Monarto plains		Volcanic plains		Other	Total	
	Jills	Does	Jills	Does	Jills	Does	Jills	Jills	Does
Total adult females	42	24	75	22	136	34	2	255	80
Age 4–12 m	17	9	27	21	72	19	1	117	49
Age >12 m	19	15	39	1	38	15	1	97	31
No lens	6	-	9	-	26	-	-	41	-
Pregnant	23	22	30	14	59	18	-	112	54
Age 4–12 m	7	8	12	13	32	10	-	51	31
Age >12 m	9	14	13	1	17	8	-	39	23
No lens	7	-	5	-	10	-	-	22	-
Abnormalities	5	2	24	2	21	-	1	51	5
Age 4–12 m	-	1	3	2	1	-	-	4	3
Age >12 m	4	1	20	-	15	-	1	40	2
No lens	1	-	1	-	5	-	-	7	-

instances of which only portion of the litter was affected, and in one fallopian tube. With the exception of one animal with adhesions and two cases of resorption, all of these hares for which eyelenses were available were >12 months old. The interaction between abnormalities (excluding resorption) and population (MP, VP, CF) was significant ($\chi^2 = 12.56$, $\chi^2_{0.005,2}=10.60$), and the interaction between abnormalities and age was also significant ($\chi^2 = 25.46$, $\chi^2_{0.001,1} = 10.83$), with a significantly higher proportion of the older adult females (52.4%, *n* = 97) being abnormal compared with the younger adult females (4.0%, *n* = 106), and a significantly higher proportion of MP hares being abnormal (36.5%, *n* = 72) compared to VP hares (13.7%, *n* = 130) and CF hares (0%, *n* = 41).

Few reproductive abnormalities were observed in the European rabbit does (see Table 4). Two instances of partial reabsorption occurred, one in a young doe and the other in an older doe; and one case of focal endometrial venous congestion was observed in a young doe. The only other abnormality involved a young doe bearing a litter with an overdeveloped (furred) foetus of 43 g, and two foetuses of 19 and 16 g with reduced numbers of digits (1–3) one of which had incomplete fusion of the abdominal cavity out of which a portion of the liver protruded.

Discussion

Breeding Season

The breeding season may be broadly defined as the period from the date of the first pregnancy to the last weaning in a population (Hansen 1992). If defined as such, the whole year would be the breeding season for hares in southern Australia. However, the definition takes no account of fluctuations in fertility through the year. Based on the clearly demarcated drop in the conception rate between May and June inclusive, the duration of the breeding season for the Australian hare sample was 8 months, extending from July to February (i.e. mid winter until late summer).

Puberty

Variation in the age of onset of female reproductive activity is a common characteristic of lagomorph populations. Puberty can be as early as 3 months of age in *Lepus capensis* (Lu 2000) and *Oryctolagus cuniculus* (Mykytowycz 1959). Broekhuizen and Maaskamp (1981) noted that female puberty in *L. europaeus* can be reached by 4 months, but in most instances is at 6 or 7 months, and sometimes later. Bray (1998) found evidence of reproduction in four female hares aged 3–4 months at the time of first mating. However, the ability to breed is also seasonally dependent, because the later the young are born during the breeding season the more likely they are to reach mature size outside of the breeding season (Martinet et al. 1970). The earliest age of conception in our study, ca. 3 months, is consistent with Bray's (1998) observations, but precocious female hares were more common in our population than in Bray's (1998) population. Hewson and Taylor (1975) suggestion that good physiological condition is important in the initiation of breeding has been confirmed by Caillol et al. (1992) who found that the age of attainment of puberty varies according to month of birth and body weight. Within the sub-adult age group, we found that parity was more closely associated with paunched body weight than with age, consistent with Lu's (2000) observations on young *L. capensis* in China, which showed that breeding females were younger but heavier than non-breeding females.

As would be expected, in lagomorphs early birth is a precondition for reproduction by a female within the season of her birth (*O. cuniculus*, Mykytowycz 1959; *L. capensis*, Lu 2000; *L. europaeus*, Lincoln 1974). Lu (2000) noted that 51.7% of *L. capensis* in China bred in the season of their birth and Flux (1967) showed that up to 70% of female New Zealand *L. europaeus* big enough to do so bred in the season of their birth; however, for *L. europaeus* in

England the proportion was very small (4%, Lincoln 1974). Thus, the contribution by sub-adult and juvenile hares to recruitment is dependent on their early season survival as leverets, their growth rate, the length of the breeding season, and the survival during the non-breeding season of their leverets which are necessarily born in the latter part of the breeding season. In Sweden, where there is high hare density, low survival of early born leverets and a short breeding season, no *L. europaeus* bred during the season of their birth (Frylestam 1980).

Reproductive Output

Lagomorphs in general have a high reproductive output because they have long breeding seasons, post-partum mating, and relatively short gestations, permitting several litters in a season; and because they are multiparous.

Several factors influence litter size in lagomorphs, including the age of the dam and the timing of the litter within the breeding season. Also, litter size may vary in the same population between years, e.g. the range of 2.06 to 2.33 observed in Danish hares by Hansen (1992), and in accordance with the nutritional status of the dam, e.g. the range of 2.6–4.9 observed in rabbits by Stodart and Myers (1966) in Australia.

Younger females tend to have smaller litters. For the European rabbit in Australia, Myers and Poole (1962) recorded a mean litter size of 4.5 in younger does, and 4.9 in older does. For the European hare, in England, Lloyd (1968) recorded lower litter sizes in primiparous hares in England. In France, Bray (1998) observed a mean litter size of 1.9 in sub-adult hares, and 2.7 in adults. A low reproductive output is to be expected in post-pubescent sub-adults because of a relative endocrine immaturity (Caillol et al. 1992). In northern France, Marboutin et al. (2003) calculated that females less than a year old contributed only 8% of reproductive output, but in our populations, females less than a year old contributed 54% of reproductive output, which we attribute to a longer breeding season and faster growth rates such that more females reach puberty in the season of their birth, and to infertility in many older hares.

In several studies, it has been shown that litters are smallest at the start of the breeding season, reach a peak in the middle of the season, and decline somewhat towards the end of the season (Broekhuizen and Maaskamp 1981; Hewson and Taylor 1975; Lincoln 1974), and this was also the case in our study.

Hence, overall mean litter size in hares is sensitive to the timing of the sample and to the proportions of the different adult age classes in the sampled population. It is dependent on whether all females are included, or only the pregnant females, and it varies between years. With these caveats, mean litter sizes in our Australian populations (2.19) were lower than those reported for European populations (2.21, Hewson and Taylor 1975;

2.48, Pépin 1989; 2.78, Marboutin et al. 2003; 2.50, Kolosov 1941; c. 2.8, Frylestam 1980).

Although litter sizes were smaller, the annual outputs of young per fertile adult female hare in the MP and VP populations were in the upper half of the range listed in the Broekhuizen and Maaskamp (1981) review of output in eight European countries, New Zealand, and Canada; and comparable with the three years of data recorded by Hansen (1992) in Denmark, although well below the figure of 13.7 reported for central France by Bray (1998). The apparent inconsistency between small litter size but average reproductive output is explained by the longer breeding season at the Australian sites, and it may be normal for hares with longer breeding seasons to have smaller litters (Flux 1967). However, the outputs per adult female (including infertile females) were in the lower half of the range.

Infertility of older hares is potentially important to hare populations, because recruitment failures can occur in some years due to unfavourable weather events (Meriggi and Alieri 1989) such that few young females are available to breed, and the older female hares which normally make a greater contribution to recruitment than females in their first year (Flux 1967) because of their longer breeding season and greater mean litter size (Frylestam 1980) would not be able to compensate. In the year following a recruitment failure, the surviving females would be older and prone to infertility, extending the impact of the event on the population. The presence of unproductive hares in breeding hare populations has been recognised in France (Pépin 1989) and Denmark (Hansen 1992), but neither author identified an age predisposition as is evident in our Australian populations.

In a New Zealand hare population, Parkes (1989) observed a distinct break in the breeding season, with no lactations occurring between late April and early July. Individual female hares in Australia have a break, but the breaks are offset such that the population breeds throughout the year, with a nadir in the autumn–early winter period of about 4 months.

The analyses of conception months and birth months rely on the method of assessment of the stage of gestation. For our study, the graph of Broekhuizen and Martinet (1979) was used, instead of the more recent graph and formula of Hackländer et al. (2003). The earlier method was used because >12% of the foetus in our sample were >108 mm CR length which corresponds to the length of gestation using the Hackländer et al. (2003) formula. Our maximum CR length was 150 mm for a litter of one, and we had three multiple litters with foetuses >140 mm. The Hackländer et al. (2003) formula will not compute beyond 122 mm CR length, where it gives a gestation of 114 days.

We considered three explanations for the discrepancy. It is possible that a genetic change has occurred in the Australian population, but because captive hares may be smaller than wild hares of the same age (Bray et al. 2002) and because there may be a maternal influence on foetal size (see Allen et al.

2002), we checked whether the Hackländer et al. (2003) mothers were small. However, their liveweight was ca. 3,400 g (Hackländer et al. 2002) whereas the mean liveweight of our hares with single foetuses above 122 mm CR was 3,253 g, and we must reject that hypothesis. We also considered whether regular handling of the captive hares in the Hackländer et al. (2003) study might have depressed foetal growth, because it has been suggested that regular handling may depress the growth rate of European rabbit kittens (Myers 1958) and also cause resorption in rabbits (Myers and Poole 1962). However, there was no evidence to support that hypothesis either, and there was no satisfactory explanation of the discrepancy.

Reproductive Abnormalities

Many of the reproductive abnormalities found in Australia have been described in other hare populations, but generally as isolated occurrences. Bensinger et al. (2000) in Germany and Hackländer et al. (2001) in Austria observed similar pathological changes in uteri of adult female European hares, all of which were older, but apparently did not examine the fallopian tubes. In the German study, as in our study, the dominant form of abnormality was cystic endometrial hyperplasia. Tubal lesions in hares in France have been reported by Pépin (1989). The proportion of Australian hares (15 out of 51 affected animals) expressing more than one of the conditions described in this work suggests that the abnormalities share an aetiology. Extra-uterine foetuses arising through uterine rupture sometimes occur at the time of parturition, in a range of species; but spontaneous rupture with subsequent survival of the mother in the absence of medical intervention is a rare phenomenon, although it has been recorded in the human (Bannerman 1965) and the sheep (Dennis 1966). In the hare however, there are at least seven reports, several of which record more than one instance (see Flux 1967; Broekhuizen and Maaskamp 1981; Stott and Wight 2004), and a British survey in progress of 416 adult female British hares has found two instances in jills >12 months of age (Jennings, N.V., University of Bristol personal comm.), suggesting that hares are particularly prone to the condition. Cystic ovarian tumours seem to be relatively common in hares, and tumours of the mammary gland and the region of the uterus have also been reported elsewhere (Flux 1965). Resorption is not regarded as a disease condition in lagomorphs as it occurs in response to social (Mykytowycz 1960) or environmental (Hewson 1976) stress. Like the Chowilla Floodplain jills, hares feeding on natural vegetation in Argentina showed no abnormalities (Bonino and Montenegro 1997). Hence, the general impression is that old female hares in agricultural areas feeding on crops and pastures suffer from a suite of reproductive problems, whilst young female hares and hares feeding on natural vegetation do not.

Although the aetiological agent(s) for the above conditions are not known, a circumstantial case can be made that the conditions are due to an external oestrogenic influence. The farms of MP and VP have leguminous pastures known to produce phytoestrogens and mycoestrogens (Reed 2001), and MP farms also have leguminous crops such as lupins. In both areas agricultural chemicals are used, some of which are known to have oestrogenic activity (Jobling et al. 1995). The hares at CF, on the other hand, have little access to introduced pasture plants, none to crops, and there is no use of agricultural chemicals in the area. Hence, there appears to be an association between the level of potential exposure to environmental oestrogens and the prevalence of infertility in hares. Further, most of the conditions listed above have been shown to have some relationship to oestrogen. Cystic endometrial hyperplasia is the major indication of phytoestrogenism in sheep (Bennetts et al. 1946), and the hare is particularly sensitive to the luteotrophic effect of exogenous oestrogen, which can double the length of pseudopregnancy in that species (Caillol et al. 1989). The formation of secretory cells in the fallopian tubes can be induced by exogenous oestrogen (Fredricsson 1959 in Odor et al. 1989). Although hares with uterine rupture are not necessarily infertile, associations between the condition and oestrogen have been made. It has been suggested that uterine rupture may be associated in humans with in utero exposure to diethylstilbestrol (Adams et al. 1989) and in sheep with exposure to phytoestrogens (Dennis 1966), and Bennetts et al. (1946) provided a connection between exposure to phytoestrogens and uterine rupture by demonstrating that the cystic development of the glands can extend through the full thickness of the myometrium, to the extent of causing spontaneous rupture in non-pregnant sheep. Welshons et al. (1987) showed that phytoestrogens can stimulate the growth of human mammary neoplasia, and fibrosis of the wall of the uterus in response to exposure to stilbestrol dipropionate has been reported in the bitch (Dow 1958). Resorption in rabbits may in most instances be attributable to stress (see above), but has also been associated with the use of oestradiol implants (Dunsmore 1971). The pattern showing only the lowest level of impact, pseudopregnancy, in younger adults and the higher levels of impact in older adults would be expected because Underwood and Shier (1951) demonstrated that the effect of phytoestrogens on sheep became progressively more severe with longer exposure, and Schinckel (1948) showed that the effect is persistent.

The absence of comparable abnormalities in sympatric European rabbits strengthens the circumstantial implication of exogenous oestrogens as the aetiological agent. Adams et al. (1981) demonstrated that the phytoestrogenic syndrome in sheep involves extension of the life of the corpus luteum in a proportion of ewes and therefore prolongation of the influence of progesterone, and that those are the sheep that exhibit pathological changes. In the rabbit, exogenous oestrogens do not prolong the life of the corpus luteum (Miller and Keynes 1976), whereas they do in hares (Caillol 1989). Hence, a pathological response by hares exposed to phytoestrogens, but not by sympatric rabbits, is consistent with our observations.

If the individual disease states described in this work are in fact part of a disease syndrome, the geographical distribution of the cases reported in the literature (above) suggests that the syndrome is widespread. Depending upon its prevalence in any particular population, the syndrome is potentially important to hare populations, because recruitment failures can occur in some years e.g. due to a sequence of unfavourable weather events (Meriggi and Alieri 1989) such that few juvenile females are available to breed, and the older jills which normally make a greater contribution to recruitment than females in their first year (Flux 1967) because of their longer breeding season and greater mean litter size (Frylestam 1980) would not be able to compensate. In the year following a recruitment failure, the surviving females would be older and prone to infertility, extending the impact of the adverse weather on the population.

Conclusion

The most distinctive feature of the demography of the hare in southern Australia is the low fertility of older hares. Independently, the fertility of the younger hares is higher than elsewhere in the world because the breeding season is somewhat longer and sub-adult puberty is more common.

Reproductive abnormalities were common in older female hares in two agricultural areas of Australia where legumes are common, but not in an area of native vegetation. We made the clinical judgement that many of the affected hares would be infertile. We have described several syndromes and noted that each has been described in isolation in the literature, and we have provided the unifying hypothesis that the syndromes are due to exposure to phytoestrogens. We conclude that infertility may delay the recovery of a hare population subjected to a year of recruitment failure.

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A Review of Competition between Rabbits (*Oryctolagus cuniculus*) and Hares (*Lepus europaeus*)

John E.C. Flux

Introduction

The concept of competition "is of no practical use for field biologists . . . But for theoretical ecology the concept is vital. On the assumption that competition occurs in nature, both between species and between individuals of the same species, it has been possible to make simple models of the natural world which have led to our most perceptive understandings of how species are separated, and kept distinct; and of how the populations of many animals and plants are restricted in nature" (Colinvaux 1973). In this review, I use the wide definition of competition to include both interference (or contest) and exploitation (or scramble) competition, because the distinction is usually obvious from the context.

If competition is very strong, the geographical ranges of the competing species may meet at a sharp boundary. Thus, four species of pocket gophers (*Geomys*, *Cratogeomys*, and *Thomomys* spp.) meet without overlap in Colorado, appearing to behave as a single species competing for space (Miller 1964, quoted by Krebs 1978). A good indicator of competition is the ability of either of two species to occupy the range of the other if it is absent, as found in the chipmunks *Eutamias dorsalis* and *Eutamias umbrinus*; although the former is dominant, the latter can out-compete it where trees are close enough to allow rapid escape (Hall 1946; Brown 1971; quoted by Krebs 1978).

Hares (genus *Lepus*) are widely distributed in grassland, steppe and desert over most continents. They are of relatively recent origin, and the approximately 30–32 species currently recognised (Flux and Angermann 1990; Chapman and Flux 2008; this book) are normally allopatric. The few cases of sympatry that have been studied, e.g. in Ireland, Finland, Russia, and Sweden, where *Lepus timidus* (Mountain hare) is endemic but *Lepus europaeus* (European hare) was introduced in the 19th and early 20th centuries, appear to be transitional, with the European hare driving the Mountain hare from the plains (Barrett-Hamilton 1912; Lind 1963; Gaiduk 1982; Thulin 2003), although Millais (1906) disagrees: "With this view I do not agree at all, for the two species are in no sense antagonistic to each other…" and "do not occupy

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the same ground as a rule, simply because their habits and food are different." (This apparent conflict seems based on misunderstanding, because Barrett-Hamilton also says: "It is certain that, although the blue hare as a species retreats before its larger relative, there is no active antagonism between the individuals of either".) Hewson (1990) observed a European hare among Mountain hares which appeared to treat it as conspecific. In Kenya *Lepus capensis* (Cape hare) and *Lepus crawshayi* feed side by side in parts of the Great Rift Valley where regular scrub fires alter the habitat to favour *capensis*, but regrowth favours *crawshayi* (Flux and Flux 1983); in Colorado *Lepus californicus* (Black-tailed jackrabbit) and *Lepus townsendii* (White-tailed jackrabbit) co-exist, using scrub and fields (Flinders and Hansen 1973); and in Mexico *L. californicus* and *Lepus callotis* (White-sided jackrabbit) co-exist on semi-desert and grassland, respectively, (Desmond 2004). Moreover, replacement of one species by another has often been recorded following climatic or anthropomorphic changes to the environment (e.g. *Lepus americanus* (Snowshoe hare) *by L. townsendii* in Wisconsin (Leopold 1947), *L. townsendii* by *L. californicus* in Kansas (Brown 1947) and *Lepus arcticus* (Artic hare) by *L. americanus* in Canada (Fitzgerald and Keith 1990, who write: "It is highly unlikely that interference competition with snowshoe hares is responsible for the current restriction of arctic hares to barrows and tundra.").

The geographic ranges of hares, however, frequently encompass those of rabbits of several genera; indeed this seems the rule except for isolated island forms like *Pentalagus* and *Nesolagus*. For example, *L. americanus* and *Sylvilagus floridanus* (Eastern cottontail), *L. californicus* and *Sylvilagus nuttallii* (Mountain cottontail), *L. townsendii* and *Brachylagus idahoensis* (Pigmy rabbit), *L. capensis* and *Pronolagus rupestri*s (Smith's red rock hare), *L. ruficaudatus* and *Caprolagus hispidus*, *L. crawshayi* and *Poelagus marjorita* (Bunyoro rabbit) (see Chapman and Flux 1990). This review examines the relationship between the European hare, *L. europaeus,* and the European rabbit, *Oryctolagus cuniculus*, the best-studied pair.

Geographic Distribution

Before human intervention *L. europaeus* was restricted to central Europe, and *O. cuniculus* to the Iberian Peninsula, where it was sympatric with *Lepus granatensis* (Liberian hare) (Flux 1994). Hence overlap between *L. europaeus* and *O. cuniculus* is relatively recent, but now widespread (because both species were important game animals) over much of Europe, South America, Australia, and New Zealand (Chapman and Flux 1990). In all areas except Australia hares occupy far more of the country than rabbits do; why hares cannot live where rabbits can in Australia remains a puzzle, but is unlikely to be due to competition because hares were liberated first and failed to spread even in the absence of rabbits (Jarman 1986).

At a regional scale it is possible to show allopatry associated with the preferred habitat of each species, and this is emphasised by most field guides: (rabbit) sandy soil, light clay, coniferous woods; (hare) cultivated land, deciduous woods (van den Brink 1967), (rabbit) dry heaths, short pasture, agricultural land close to cover, edges of pine woods; (hare) cereal fields, pasture, woods and shelterbelts (Corbet and Harris 1991), (rabbit) sunny aspect, light soil, and adequate cover; (hare) sand dunes, cropland, pasture, clearings in scrub or forest (King 1990).

On the local scale, also, there is a very old and widespread opinion among shooters and country folk that hares and rabbits avoid each other: "The hare is essentially a fastidious animal. Like sheep, it refuses to graze on grass lands which rabbits have defiled" (Macpherson 1896). "Field naturalists are unanimous that the animals are naturally antipathetic, and will not even thrive well on the same ground. This may be because rabbits, when in numbers, bully, chase and worry hares to death, or, perhaps, because the rabbits eat or taint the food of the more delicately feeding hares, or because the former infect the latter with epidemics to which they are themselves immune"(Barrett-Hamilton 1912). Similar views are found throughout Europe (Fraguglione 1960); in Australia after rabbits arrived Creed (1917) recorded: "and now a hare is never seen except in large areas of fenced country"; in New Zealand: "hares are not seen where rabbits are found in any numbers and vice versa" (Cox 1976) and in Chile, South America, they "do not occur in the same areas because the hares drive the rabbits away" (Housse 1953). In Argentina there are reports of "a similar process with the "tapeti" (*Sylvilagus brasiliensis*) which was replaced by the hare in only six years" (Grigera and Rapoport 1983).

Yet in all these places hares and rabbits can be found grazing side by side, and several studies of their diet show major overlap (e.g. Homolka 1987; Chapuis 1990): indeed, more overlap than the diets of some allopatric species of *Lepus* where their ranges meet (Flux 1970; Stewart 1971).

Myxomatosis

The removal of rabbits by myxomatosis in Britain and much of Europe in the 1950s provided a natural experiment, and there were early reports of increases in hare numbers in England (Moore 1956; Rothschild and Marsh 1956) and France (Rothschild 1958) as would be expected if rabbits and hares were in competition. However, more detailed analysis of a French shooting estate (Birkan and Pepin 1983) showed this increase was short-lived, and the hare and rabbit populations were responding to different climatic factors. In Britain, similarly, field observation, and an analysis of extensive game bag records, led Barnes and Tapper (1986) to suggest that the increase in grass cover may have allowed higher leveret survival; and the decline in hare numbers since the 1960s "appears to be quite unrelated to the recovery of the rabbit population" but "how hares benefit from the absence of rabbits is not properly understood" (Boag and Tapper 1992). In Hungary, Katona et al. (2004) found no increase in hares when disease eliminated a dense rabbit population from their study area in 1994–95 and concluded that the competitive effect of rabbit on sympatric hares had been low or expressed in some unstudied factors. In Australia, according to Frith (1970) "There seems little doubt that since the reduction of rabbit populations (by myxomatosis in 1950) the numbers of hares have increased in some places and this trend seems to be continuing". New Zealand introductions of myxomatosis failed, but following the illegal introduction of RHD in 1997, the number of hares shot has increased (G. Norbury pers. comm.). None of these correlations, however, give any indication of the mechanism involved.

Behavioural Observations

Antagonism between rabbits and hares has often been reported, both in captivity and in the wild. Gayot (1868) bred hares successfully in cages, and found rabbits mutilated hares. This was also reported by Thierry (1907) and Kunstler (1908). In Britain, in the wild, a rabbit was watched attacking a hare until it screamed (Booth 1883, quoted by Barrett-Hamilton 1912). Rabbits "are sufficiently bold to drive the hares before them. This can easily be verified by observation in the early hours of the day, in any place where both species happen to be numerous" (Macpherson 1896). Millais (1906) states that "Rabbits have the greatest objection to Hares, and will give them no peace until they have driven them away from the neighbourhood of their burrows."

More recent scientific observations, however, appear to indicate that overt antagonism is very rare. On the Isle of Wight, England, hares and rabbits fed together peacefully, leading Moore (1956) to ask: "How many of us have seen hares and rabbits fighting? I never have done, and would be interested to know if anyone else has." In a classic study in the Netherlands, Broekhuizen (1975) analysed 1,241 meetings between rabbits and hares: only in 1% was there an attack, "sometimes by the hare, mostly by the rabbit"; and because there were many rabbit vs. rabbit attacks, he suggested that some rabbit vs. hare attacks may be mistakes. He concluded "that hares are not driven out by aggressive behaviour of rabbits, that hares do not avoid land used by rabbits and that they do not avoid rabbits in general." In southern England, Barnes and Tapper (1986) counted by spotlight on nine arable farms in spring and autumn of 2 years. They found rabbits and hares were positively associated on cultivated land in spring, and on stubbles in autumn, with no evidence that hares avoid rabbits: "Nor have we seen any evidence of competition; in general rabbits feed along the edges of fields while hares are more likely to be in the middle of the same fields", although there was some evidence of a reduction in hare numbers where rabbit density was very high.

In New Zealand, when rabbit populations were severely reduced by poisoning in the 1950s, farmers and professional rabbit-control personnel noted a widespread increase in hares, and I was appointed to study the problem in 1960. An area of 3 ha in Travers Valley, Nelson Lakes National Park had a resident population of about 25 rabbits that declined to zero in 2 years. It was also the main feeding ground for about ten hares, and their interactions were recorded for a week once a month for 2 years. Most rabbits and hares ignored each other and often fed within 1 m of each other. The totals of the chases seen in 2,314 observed encounters were: hare vs. hare, 142; rabbit vs. rabbit, 155; and hare vs. rabbit, 55, of which 45 were of rabbits chasing hares (Flux 1981).

Since 1972 I have recorded all hares and rabbits seen from our house overlooking a 3-ha area of uniform sheep pasture, later used for horse grazing. Both rabbits and hares were present at extremely low densities, and months could elapse without any being seen. Over 32 years of observation, the number of rabbits has increased as they have over most of New Zealand since the rabbit control organisation was disbanded in 1981 (Flux 1997). Grouped in 8-year totals, the proportion of rabbits to hares was 1/67 (1.5%), 21/67 (31%), 10/22 (45%) and 59/98 (60%). Group sizes for the two species were similar, averaging 1.32 (1–5) for rabbits and 1.36 (1–5) for hares. In contrast, the corresponding figures for the high density populations in Travers Valley were 3.39 (1–17) and 1.91 (1–8). At Belmont, the totals of chases seen in 265 observations were: hare vs. hare 47, and rabbit vs. rabbit 17; hares and rabbits were seen together 16 times, but without any interaction at all.

Shared Diseases

The idea that rabbits carry diseases to which hares are more susceptible has a long history reviewed by Barrett-Hamilton (1912). Broekhuizen (1975) investigated this in the Netherlands and concluded that *Graphidium strigosum* was an original parasite of rabbits that could harm hares entering areas where rabbits were abundant, but more information was needed to verify a causal relationship. In Britain, Barnes and Tapper (1986) considered the infection rates too low to be involved. The concept of competition via host-parasite relationships is well discussed by Barbehenn (1969), who suggested that *Sylvilagus* could be introduced to spread myxomatosis to *Oryctolagus*, but "The Australians would have lined the shores with shotguns to keep out another rabbit." The experiment has now been carried out in Europe with successful introductions of *S. floridanus* since 1966. Only in Piedmont, Italy, did it spread rapidly, apparently because it faced little competition there (Spagnesi and De Marinis 2002).

Competition on Islands

Van Laar (1981) reviewed the history of mammal introductions on the Wadden Sea islands, the Netherlands, and considered that hare competition excluded rabbits on Pellworm, and rabbits excluded hares from Vlieland until the construction of polders made the area more suitable for hares.

Rabbits have been liberated on more than 800 islands worldwide (Flux and Fullagar 1992) and data are available on the fate of the populations on 607 of these islands, analysed by Flux (1993). The rabbits died out, presumably because of adverse environmental conditions, on 11.2% of these, and this mortality was not related to the island size. Myxomatosis removed rabbits from 10.1% of 119 islands, more efficiently from smaller islands, and not from any of over 1,000 ha. Cats removed rabbits from 11.3% of 80 islands, including 7% of the islands in the 1,000–10,000 ha category. Hares, however, were far more efficient at removing rabbits, clearing 26.7% of 105 islands, including 5% of those in the 100,000+ ha category. These figures appear to indicate that the force of competition is relatively twice as strong as predation by cats; or by myxomatosis, which can kill 99% of a susceptible rabbit population.

Discussion

European hares and European rabbits became sympatric relatively recently, and they occupy agricultural or pastoral habitats which are also of recent origin. Hence one explanation of the rather unusual interspecific aggression historically reported as displayed by rabbits to hares is that they were in the process of establishing or re-defining their ecological niches.

The high level of interspecific aggression recorded by early writers was not supported by careful observers like Moore (1956) and Broekhuizen (1975). This discrepancy could be explained by the far lower densities of rabbit populations following myxomatosis. European hare populations are also lower throughout Europe than they were 100 years ago (Niethammer and Krapp 2003), so the chances of observing aggression is much reduced; indeed the reason for it may no longer exist.

Their diets when studied in the same locations are remarkably similar (Homolka 1987; Chapuis 1990), and Wolfe et al. (1996) suggest that the species can co-exist by avoiding competition in spatial use, as proposed by Hulbert et al. (1996) for mountain hares and rabbits. I find this unconvincing, because both species will occupy most of the habitat of the other in its absence. However, as the two species operate at a different scale, their overlap or avoidance may depend on the interaction between the structure of the vegetation (patch size) relative to their very different home range sizes (V. Altbäcker in lit. 2005).

On islands, individual species of vertebrates reach higher densities than on the adjacent mainland, and this holds for hares (e.g. Angerbjorn 1981). There are also more species on large islands than on smaller ones. These relationships would affect the outcome of liberations of rabbits and hares on islands, but there appear to be no behavioural studies of interactions or aggression. Because either species may take over the island depending on the balance of favourable factors (van Laar 1981), it seems unlikely that a disease is involved to which one species is more susceptible, as suggested by Barrett-Hamilton (1912), and Broekhuizen (1975).

Other genera of rabbits and *Lepus* species seem to co-exist without aggression, and there is no popular belief that jackrabbits and *Sylvilagus*, for example, are antagonistic. Orr (1940) often "observed jack rabbits feeding within a few feet of both brush rabbits and cottontails. No animosity was ever apparent between these species at such times". European rabbits, perhaps because they are feral domesticated stock, reach population densities with a biomass about ten times higher than any other lagomorphs (Flux 2001). This certainly explains why they can degrade the vegetation and make it unsuitable for hares (Fraguglione 1960), but not how hares are able to remove rabbits from islands so efficiently, unless rabbit populations overeat the food supply and are basically unstable (Wynne-Edwards 1962).

The mechanism involved in maintaining allopatry in different species of *Lepus* is unknown, but must be remarkably strong to result in such clear and relatively fixed boundaries. An individual European hare, for example, is easily capable of traversing the entire range of *Lepus castroviejoi* (Broom hare) in a few hours, yet apparently never does. But aggression seems not to be involved. It is likely that the same or a very similar mechanism is used in interspecific competition between European hares and European rabbits: in both cases, the species in contact seldom show aggression and, if they do, the level of interaction is of the order expected for intraspecific conflicts. The resolution of this problem could be the key to population regulation in lagomorphs.

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Diseases

European Brown Hare Syndrome

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Introduction

European brown hare syndrome (EBHS) is a highly contagious, acute disease of the European hare (*Lepus europeaus*) and mountain hare (*Lepus timidus*) first described in the early 1980s in Northern Europe (Gavier-Widén and Mörner 1991). The aetiology of EBHS remained unclear until it was shown through animal experiments and electron microscopy (EM) analysis (Eskens and Volmer 1989; Lavazza and Vecchi 1989) that it is caused by a non-enveloped positive-strand RNA virus with a diameter of about 32–35 nm, showing morphological characteristics indistinguishable from those of the rabbit haemorrhagic disease virus (RHDV) (Capucci et al. 1991) and biochemical features typical of the Caliciviridae family (Wirblich et al. 1994). EBHSV is composed of a single-stranded RNA of about 7.5 kb and a subgenomic RNA of 2.2 kb in length. It has a single major capsid protein of approximately 60 kD that shares epitopes with the capsid protein of RHDV (Wirblich et al. 1994) and haemagglutinates human group O red blood cells (Gavier-Widén and Mörner 1991).

Due to the existence of many similarities with regard to aetiology, epidemiological data and clinical-pathological features, at least initially, EBHS and RHD were considered as the same disease caused by a single agent. However, significant antigenic, structural, and molecular differences between the two viruses were found using RHDV-monoclonal antibodies (MAbs) (Capucci et al. 1991, 1995), cross-hybridisation and genomic sequence analysis (Wirbilch et al. 1994). Alignment of the RNA sequences of the EBHSV and RHDV genomes reveals 71% nucleotide identity, and amino acid alignment reveals 78% identity and 87% similarity (Le Gall et al. 1996).

All known EBHSV isolates appear to belong to one serotype. The complete sequence of geographically different EBHSV strains has been reported and compared by phylogenetic analysis (Nowotny et al. 1997; Le Gall et al. 2004). This

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analysis revealed close overall homology in terms of genome sequence (maximum nucleotide divergence of 11.7%) indicating a high level of conservation between isolates. On the contrary, the antigenic characterisation using a panel of 13 different specific MAbs, which recognise at least nine epitopes exposed on the surface of the virus, can indicate the existence of different viral strains (Lavazza et al. 1999).

Host Range

European hares and Mountain hares are susceptible. No other species are known to be affected, either naturally or experimentally. Recent studies attempted to demonstrate the susceptibility of cottontail rabbits (*Sylvilagus floridanus*) to EBHSV showing seropositive reactors in a free-ranging population. In addition, it was possible to induce clinical disease and mortality in a low number of experimentally infected animals (Tizzani et al. 2002).

In consideration of the close antigenic and genomic correlation existing between RHDV and EBHSV several experiments have been conducted in order to ascertain the reproducibility of both diseases in the heterologous species. However, the experimental inoculation of rabbits and hares with the reciprocal virus does not result in virus replication and stimulates only a slight, non-protective antibody response (Lavazza et al. 1996; Lenghaus et al. 2001).

Distribution, History and Evolution

European hare syndrome has been reported in many European countries: Germany (Eskens and Volmer 1989), Italy (Lavazza and Vecchi 1989; Poli et al. 1991), Belgium (Okerman et al. 1989), United Kingdom (Chasey and Duff 1990), Croatia (Sostaric et al. 1991), Sweden (Gavier-Widén and Mörner 1993), Finland (Salmela et al. 1993), Austria (Steineck and Nowotny 1993), Spain (Gortazar and de Luco 1995), Poland (Frölich et al. 1996), Switzerland (Frölich et al. 2001), Slovakia (Frölich et al. 2007) (see Fig. 1).

However, it was not known outside Europe (Lenghaus et al. 2001) until 2003 (Frölich et al. 2003). EBHS occurred in Europe many years before the appearance of RHD. In fact, the earliest confirmed case of an apparently "new disease" called European brown hare syndrome, of which hunters had been aware since the 1970s, was from 1981 in Sweden (Gavier-Widén and Mörner 1991). The origins of these viruses are difficult to trace. They may have mutated from an avirulent calicivirus of Eurasian lagomorphs (Rodák et al. 1990; Capucci et al. 1996) or by the introduction of a new virus avirulent in its native host, possibly South American rabbits (*Sylvilagus* sp.)

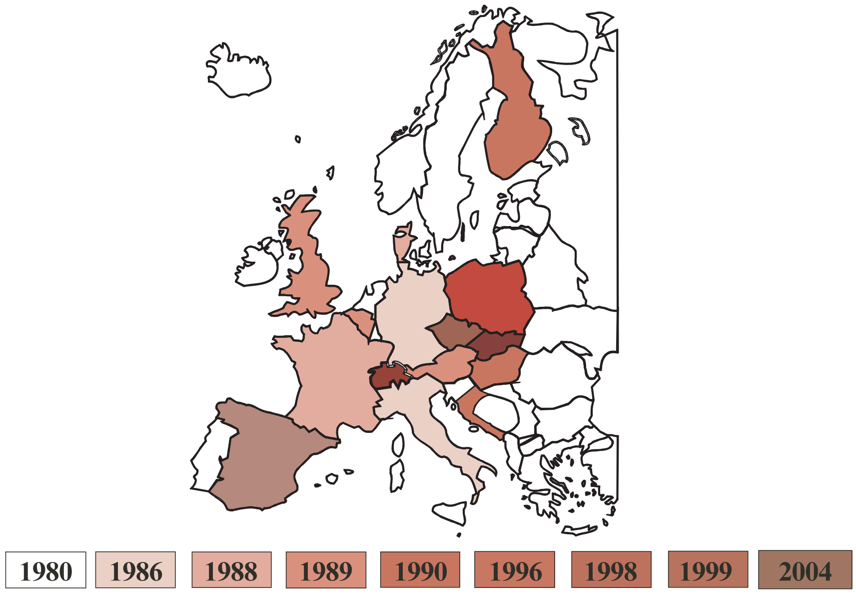

Fig. 1 Temporal distribution of diagnosed European brown hare syndrome (EBHS) in Europe (only published data)

or hares (*Lepus* sp.) imported into Europe in large numbers for recreational hunting in the 1970s and 1980s (Cancellotti and Renzi 1991). Positive identification of EBHSV in European hares from Argentina has been shown either directly by virus identification (Frölich et al. 2003) or indirectly by serological evidence of specific antibodies (Lavazza et al. 1992; Scicluna et al. 1994; Lavazza and Capucci 1996). It is assumed that a less pathogenic form of EBHSV may have been carried from Europe to Argentina in the 19th century by the importation of European hares and remained apathogenic until now. This hypothesis is supported by the following studies: Bascunana et al. (1997) reported detection of EBHSV-antigen by polymerase chain reaction in paraffin embedded specimens collected in the 1970s in Sweden. Lesions consistent with EBHS have been described from England since 1976 (Duff et al. 1994) and specific antibodies were found in sera archived since 1962 (Duff et al. 1997), although confirmed clinical cases of EBHS were first diagnosed by pathological and EM investigations in the UK in 1990 (Chasey and Duff 1990). This demonstrates that EBHSV might have occurred in European hare populations years before clinical signs of EBHS were described. In conclusion, it is conceivable that a less pathogenic variant of EBHSV may exist among European hares in Argentina and that an ancestor of the present European EBHSV strain might have been apathogenic (Frölich et al. 2003).

Clinical Signs and Pathology

The clinical signs and the gross and microscopic lesions observed in hares affected by EBHS are very similar to those described in RHD in rabbits. The disease in hares, even in its acute evolution, lasts slightly longer and causes a lower mortality rate (around 50%) than RHD in rabbits; the peak of mortality in experimentally infected hares is commonly observed between 72 and 90 h after infection. Death can be sudden, lacking any clinical signs. More often, however, behaviour modifications could be observed such as lack of fear, dullness, jumping into the air, circling, staggering, incoordination, and convulsion before death (Duff et al. 1994). In farmed hares, it is also possible to observe anorexia, apathy alternated with excitement, cries, and respiratory distress during agony. During an outbreak, around 30–50% of hares may show a chronic or subclinical course of the disease characterized by generalised jaundice clearly visible at a mucosal and subcutaneous level. Such affected hares can die after several days or finally recover. At necropsy, the principal findings are oedema and congestion of tracheal mucosa with foamy haemorrhagic contents, liver enlargement, degeneration and decolouration with sharply demarcated and friable lobes, enlargement of the spleen, and generalised jaundice (Capucci et al. 1991; Duff et al. 1994).

Transmission, Epidemiology and Impact on Populations

EBHSV is a highly contagious disease transmitted directly or indirectly, mainly by orofaecal and respiratory routes. Humans, insects, and birds can act as vectors but no reservoir hosts have been yet identified. Infection via consumed vegetation is also likely, with the virus being secreted and excreted, and also being spread in the droppings of predators that have consumed infected hares. EBHSV is highly robust, resisting acid of pH3, and may remain infectious for 3–4 months in the field.

The disease has not been observed in hares younger than approximately 40–50 days and hares 2–3 months of age may contract infection but usually do not develop clinical disease. Mortality is highest in the autumn when the population is most dense and the young of the year become susceptible (Scicluna et al. 1994; Lenghaus et al. 2001).

Even though a decline in the number of hares can be attributed to outbreaks of EBHS, the infection, after the initial epidemic peak tends to endemicity. Areas where EBHS is endemic appear to maintain a stable hare population in which most hares are immune and mortality rates are low. In highly endemic areas, like Italy for example, 95% of the free-ranging European hares have antibodies against EBHSV (Lavazza et al. 1992; Scicluna et al. 1994).

The impact on local hare populations can vary from 7 to 90% mortality (Löliger and Eskens 1991). Under certain circumstances, local European hare populations can be reduced dramatically. In fact, the impact of the infection on the population dynamic can be dramatic when the hare density is low (<8 hares/km^2). The mortality can therefore be reduced and attain endemic stability by increasing the host density (about 15 adults/km^2) (Lavazza et al. 1999).

Virological Diagnosis

The liver contains the highest viral titre and is the organ of choice for identification of EBHSV. The amount of virus present in other parts of the body is directly proportional to vascularisation; thus the spleen and serum are quite rich in the virus and can serve as alternative diagnostic materials. In particular, higher levels of subviral particles can be detected in the spleen than in the liver of those animals that died from a subacute or chronic form of the disease.

As no satisfactory growth conditions and sensitive cell substrates have been established, in-vitro isolation of EBHSV cannot be included among the virological methods (Gavier-Widén and Mörner 1991; Wirblich et al. 1994). Inoculation therefore remains the only way of isolating, propagating, and titrating the infectivity of the virus (Capucci et al. 1991).

To demonstrate haemagglutination (HA) activity in organs from EBHSV-infected hares, a modified procedure of the RHDV HA protocol should be adopted: all steps are carried out at 4°C, the organ suspension is treated with chloroform, and human group O RBCs are used at a pH 6.5. Even when using this method, only about 50% of the samples give positive results because the disease is often subacute or chronic and therefore the virus has the antigenic and structural characteristics typical of the degraded particles (Capucci et al. 1991).

EBHSV can also be identified in diagnostic samples by EM examination. In particular, the immune-EM method using convalescent anti-EBHSV serum or specific anti-EBHS MAbs can be used to identify EBHSV.

For diagnosis of EBHSV it is possible to use the RHDV-specific sandwich ELISA, but, due to the high antigenic difference existing between lagomorph caliciviruses, there is a risk of obtaining false-negative results. Therefore, the adoption of an EBHSV-specific sandwich ELISA technique using either a high-titre positive anti-EBHSV hare serum, or cross-reacting RHDV MAbs, or specific EBHSV MAbs is highly recommended. Such ELISA in association with a panel of MAbs is also useful for antigenic characterisation of different viral strains (Lavazza et al. 1999).

Western blot analysis can also be used to identify EBHSV. The pattern of protein bands, detected using either an anti-EBHSV polyclonal serum or cross-reacting anti-RHDV MAbs, is similar to that of RHDV. However, the percentage of samples showing viral degradation is higher, and therefore several fragments of lower molecular weight, originating from the VP60 structural protein, are often observed (Capucci et al. 1991).

RT-PCR appears to be an extremely sensitive method for the detection of EBHSV and it is 10^4-fold more sensitive than ELISA. However RT-PCR is not strictly necessary for routine diagnosis. It is more appropriate for investigations on molecular epidemiology to study the pathogenesis of the infection and to detect virions in young animals at the time they get infected and are not diseased (less than 2–3 months of age), in non-specific hosts (other vertebrates) and in vectors (mosquitoes and fleas).

Immunity and Serological Diagnosis

Infection by EBHSV can be diagnosed through detection of a specific antibody response. As the humoral response has great importance in protecting animals from EBHSV, determination of the specific antibody titre predicts the ability of rabbits to resist EBHSV infection. Newborn hares may have antibodies against EBHSV, indicating that there is colostral or transplacental passive transfer of immunity (Gavier-Widén and Mörner 1991). Hares that survive infection develop long-lasting, protective immunity (Lenghaus et al. 2001). Seroconversion could also be demonstrated after vaccination of hares with specific vaccines.

Due to the significant antigenic differences existing between RHDV and EBHSV, the serological techniques, which use RHDV as antigen, are not recommended for the serological diagnosis of EBHSV. However, a direct ELISA method could be employed for the detection of positive and negative EBHSV hare sera; in fact, the adsorption of RHDV on to the solid phase of an ELISA microplate exposes cross-reactive internal antigenic determinants. Alternatively, a specific competition-ELISA for EBHSV can be arranged using specific antigen and antisera (Capucci et al. 1991; Scicluna et al. 1994). These two methods have different characteristics: the solid phase ELISA is highly sensitive and low specific and able to detect a wider spectrum of antibodies whereas the competition ELISA has a high specificity but a low sensitivity since it mainly measures antibodies, binding with high avidity, directed against antigenic determinants on the viral external surface. By the combined use of the two methods differences in absolute values of titres were found while testing sera taken from hares living in areas where the disease is not commonly detected (esp. South America and Africa). This is considered a primitive indication of the possible existence of EBSHV-like particles different from virulent EBHSV (Lavazza and Capucci 1996).

Treatment and Control

There is no specific treatment for EBHS. Antiserum taken from convalescent or hyperimmunised hares given early during the incubation period may prevent death.

There are no commercial vaccines for hares, but when EBHS mortality sets off in a farm, autogenous killed virus vaccines can be produced from livers of the hares that died first in order to immunize the remaining apparently unaffected hares. In the wild, following introduction of EBHSV, spread of the disease to the naïve population in contiguous areas is inevitable. Eradication of EBHS in a wild population of hares is not feasible. Translocation of infected hares into non-endemic areas should be avoided. At hare farms, prevention is based on quarantine, serologic testing of new hares, and elimination of contact with wild hares and predators (cats, feral dogs, and foxes) by fencing. The opportunity for indirect infection through grass or hay contaminated with excreta from wild hares or through contaminated clothes or shoes should be eliminated (Lenghaus et al. 2001).

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How Many Caliciviruses are there in Rabbits? A Review on RHDV and Correlated Viruses

Antonio Lavazza* and Lorenzo Capucci

Introduction

Rabbit haemorrhagic disease (RHD) is a highly contagious and acute fatal disease of the European rabbit (*Oryctolagus cuniculus*), caused by a calicivirus (RHDV). It was first reported in 1984 in the People's Republic of China (Liu et al. 1984); currently, RHD is diffused worldwide, and has been reported in over 40 countries. It is presently endemic in Asia, Europe, Central America and Oceania. Outbreaks have also been recorded in Saudi Arabia and West and North Africa. In 2000 and 2001, three independent outbreaks were recorded in the USA and also in Uruguay at the end of 2004.

General Aspects of RHD

Host Range

The European rabbit (*O. cuniculus*) is the only species affected by RHD. Other lagomorphs, such as the Volcano rabbit of Mexico (*Romerolagus diazi*), the Black-tailed jackrabbit (*Lepus californicus*) and the Eastern cottontail (*Sylvilagus floridanus*) of North America have been shown to be not susceptible (Gregg et al. 1991).

A similar disease, termed European hare syndrome (EBHS), has been described in the European hare (*Lepus europaeus*), but its aetiological agent, which is also a calicivirus, is different from RHDV, although it is related genetically (71% nucleotide identity) and antigenically due to the existence of common internal epitopes (Wirblich et al. 1994). Cross infection does not occur by experimental infection of rabbits with EBHSV and hares with RHDV (Lavazza et al. 1996). Although some authors have suspected a possible passage to humans (Smith et al. 1998), no data currently support this hypothesis (Greenslade et al. 2001).

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Epidemiology

RHD is characterised by high morbidity and a mortality rate between 40 and 90% (Xu and Chen 1989). Infection occurs in wild and domestic rabbits of all ages, but clinical disease is observed only in adult breeders and young animals older than 40–50 days. The mechanism of resistance in young animals is still unclear and is probably correlated to both the pathogenesis of the infection and the physiological development of rabbits. Some hypotheses on this mechanism are reported by Cooke (2002).

RHD spreads very rapidly and infection can occur by nasal, conjunctival or oral routes. The disease could be transmitted directly from infected animals to healthy ones or indirectly by contact with a carcass, or due to contamination of feedstuffs and water by excretions and secretions of infected animals (Xu and Chen 1989). Transmission by passive vectors, such as insects, birds and rodents, or contaminated utensils and vehicles can also occur (Xu and Chen 1989; Chasey 1994; Asgari et al. 1998). Man seems to play an important role as a passive vector of the virus by passing the disease from one rabbitry to another (Xu et al. 1985). The fur from diseased rabbits has been shown to carry the virus (Chen 1986) and carnivores fed with infected carcasses can excrete the virus in faeces at infectious titres (Simon et al. 1994).

Clinical Signs

The clinical evolution of the disease can be peracute, acute, subacute or chronic (Xu and Chen 1989; Marcato et al. 1991). The clinical manifestations have been described mainly in the acute infection, as there are usually no clinical signs in the peracute form, and the subacute form is characterised by similar but milder signs. The incubation period varies between 1 and 3 days; death may occur 12–36 h after the onset of fever (>40°C). During this phase, various signs could be observed such as anorexia, apathy, dullness, prostration, nervous signs (convulsion, ataxia, paralysis, opisthotonos, paddling), groans and cries, respiratory signs (dyspnoea, frothy and bloody nasal discharge), cyanosis of mucous membranes (Marcato et al. 1991). During an outbreak, a limited number of rabbits (5–10%) may show a chronic or subclinical evolution of the disease, which is characterized by severe and generalized jaundice, loss of weight and lethargy. These animals often die 1 or 2 weeks later, probably due to liver dysfunction. In meat animals, the hepatic lesions (degeneration and necrosis) in subclinical and chronic cases may be evident only during meat inspection at slaughter (Barbieri et al. 1997; Capucci et al. 1991).

Lesions

Due to the rapid course of this disease, the animals are usually found in good condition after death. The gross pathological lesions are variable and may be subtle, and include circulatory and degenerative disorders (Marcato et al.

1991). Liver necrosis and splenomegaly are the primary lesions. The liver appears yellowish-brown in colour, brittle and degenerated, with a marked lobular pattern. The tracheal mucosa is hyperaemic and contains abundant frothy fluid and the lungs are oedematous and congested. A massive coagulopathy is usually the cause of haemorrhages in a variety of organs and sudden death. In subacute and chronic disease, an icteric discoloration on the ears, conjunctiva and subcutis is clearly evident.

The microscopic hepatic lesions are of great diagnostic importance. There is multifocal to massive coagulative hepatic necrosis, often in midzonal or paracentral areas. Inflammatory infiltrate is mild to moderate and consists of lymphocytes in portal spaces and sinusoids and granulocytes in sinusoids. Tracheal and pulmonary lesions are mainly of the hyperaemic-oedematous type. In the lung, there are locally extensive haemorrhages into alveoli. Lesions in the spleen vary from simple congestion to necrosis and haemorrhage in the white and red pulp. Eosinophilic fibrin microthrombi can be seen in the vasculature of most organs (Marcato et al. 1991).

The Causative Agent of RHD: Identification and Diagnosis

Aetiology

The causative agent of RHD is a calicivirus (genera Lagovirus) that is 32–35 nm in diameter and has a single major capsid polypeptide (60 kDa), a positively stranded RNA genome of 7,437 kb and a sub-genomic RNA of 2.2 kb (Capucci et al. 1990; Meyers et al. 1991a, 1991b; Ohlinger et al. 1990, Parra and Prieto 1990). The RHD virus (RHDV) capsid protein (VP60) folds in two distinct domains held together by a hinge region: the N-terminal 200–250 residues constitute the inner domain and the C-terminal residues beyond 200–250 constitute the protruding domain. In the overall picture of the capsid, these domains form the inner shell and the outer shell, respectively, which is characterised by arch-like structures. This structure also correlates with the antigenic characteristics of RHDV, in fact the main antigenic determinants are located on the C-terminal end of the VP60 (Capucci et al. 1995a, 1998; Schirrmaier et al. 1999; Wirblich et al. 1994).

A second type of virus particle (s-RHDV) is commonly found as the main component in approximately 5% of the RHDV-positive specimens, i.e. those taken from rabbits showing a protracted course of the disease (Barbieri et al. 1997; Capucci et al. 1991; Granzow et al. 1996). The characteristics of this particle are: (i) a smooth surface and a diameter smaller than RHDV; (ii) one protein of 28–30 kDa that corresponds to the N-terminal part of the VP60; (iii) reactivity with RHDV convalescent rabbit sera and with those anti-RHDV monoclonal antibodies (MAbs) reactive against the N-terminal end of the RHDV VP60; and (iv) lack of haemagglutinating properties (HA negative). This s-RHDV particle corresponds only to the inner shell of RHDV and large amounts of it can be detected only in the liver and spleen but not in the bloodstream, especially from 3–4 days post-infection, when specific anti-RHDV IgM are appearing. These data, in association with the finding of fragments of the VP60 having different molecular weight (41–30 kDa), during transition from RHDV to s-RHDV led Barbieri et al. (1997) to concluded that the genesis of the particle is due to a degradative process that is probably the consequence of the physiological clearance of the RHDV-IgM immuno-complex formed in large amounts at the beginning of the humoral response. Therefore the identification of this second particle in the liver of a rabbit can be considered as a marker of the subacute/chronic form of RHD that usually evolves between 4 and 8 days post-infection and is followed either by the death of the rabbit or, more often, by its recovery (Barbieri et al. 1997).

Virus Properties and Resistance

RHDV is very stable and resistant in the environment; viral infectivity is not reduced by treatment with ether or chloroform and trypsin, by exposure to pH 3.0, or by heating to 50°C for 1 h. The virus survives at least 225 days in an organ suspension kept at 4°C, at least 105 days in the dried state on cloth at room temperature, and at least 2 days at 60°C in both organ suspension and in the dried state (Smid et al. 1991). Recent studies indicate that RHDV can survive in rabbit carcasses for at least 3 month, while viral particles exposed directly to environmental conditions are viable for a period less than a month (Henning et al. 2005). It also retains its infectivity at low temperatures and remains quite stable during freezing and thawing. RHDV is inactivated by 10% sodium hydroxide, by 1.0–1.4% formaldehyde and by 0.2–0.5% beta-propiolactone at 4°C. Such treatments do not alter the immunogenicity of the virus (Arguello 1991; Xu and Chen 1989).

Virological Diagnosis

The peracute course of the disease is the most salient feature in seronegative rabbit populations. This clinical feature, in association with respiratory distress, high mortality, and rapid spread, is sufficient for a presumptive diagnosis of RHD.

The liver contains the highest amount of virus and is the organ of choice to submit for viral identification. The amount of virus present in other parts of the body is directly proportional to vascularisation; thus the spleen, lungs and serum are quite rich in the virus and can serve as alternative diagnostic material.

As no satisfactory growth medium or sensitive cell substrates have been established for culture of RHDV, in vitro isolation of virus cannot be included among the virological methods. Tissue suspensions of organs are directly examined with one of the diagnostic methods established, among which those commonly used for routine examinations are:

- *Sandwich ELISA test using RHDV specific Monoclonal Antibody (MAb)* (Capucci et al. 1995a; Capucci and Lavazza 2004).
- *Sandwich ELISA test using a panel of RHDV specific MAbs.* This test permits the identification of RHDV variants, and particularly to distinguish between the original RHD virus and its first consistent antigenic variant RHDVa (Capucci et al. 1998).
- *Western blot analysis using RHDV-MAbs* that recognize internal epitopes and also cross-reactive with EBHSV (Capucci et al. 1991). It is usually performed on the few samples that give doubtful results in ELISA test and in animals that die due to the "chronic" form of the disease.

Additional tests (Capucci and Lavazza 2004) that either have been extensively used in the past or are currently employed only for particular investigations are:

- *Negative staining ImmunoElectronMicroscopy and ImmunoGold* using both MAbs and rabbit and hare hyperimmune sera (Lavazza and Capucci 1993);
- *Haemoagglutination tests* using human type O erythrocytes (Liu et al. 1984; Capucci et al. 1996a);
- *Reverse transcription polymerase chain reaction (RT-PCR)* (Gould et al. 1997; Guittre et al. 1995). This is an extremely sensitive method for the detection of RHDV and it is 10^4-fold more sensitive than ELISA. However, RT-PCR is not strictly necessary for routine diagnosis but it is more appropriate for investigations on molecular epidemiology to study the pathogenesis of the infection and to detect virions in young animals at the time they get infected and are not diseased (less than 40–50 days of age), in non-specific hosts (other vertebrates) and in vectors (mosquitoes and fleas).

Other diagnostic methods have been developed including: immunostaining of paraffin embedded sections (Stoerckle-Berger et al. 1992), fluorescent antibody test on tissue cryosections (Gregg 1992) and in situ hybridization (Gelmetti et al. 1998).

Immunity and Serological Diagnosis

Animals that overcome the disease present a striking seroconversion that can be easily detected 4–6 days after infection. Indeed, as the humoral response has great importance in protecting animals from RHD, determination of the specific antibody titre after vaccination or in convalescent animals is predictive of the ability of rabbits to resist subsequent RHD virus infection.

Three basic techniques are applied for the serological diagnosis of RHD virus: haemagglutination inhibition (HI), indirect ELISA and competitive ELISA.

The tests used for routine diagnostic work include:

- *Competition Elisa (cELISA)* using MAbs that is considered the standard and reference test for RHD. It is highly specific since it mainly measures antibodies directed against antigenic determinants on the external surface of the virus, usually the most specific and functionally important (Capucci and Lavazza 2004).
- *Sandwich ELISA developed using anti-isotype MAbs (isoELISAs or IgElisa)* to test the sera for the presence of specific anti-RHDV IgM, IgA and IgG. The isotype titres could be critical for the interpretation of field serology and for correctly classifying the immunological status of rabbits (Cooke et al. 2002).

Some other additional tests could be used for particular investigations and particularly when a higher level of sensitivity is needed in order to detect antibodies in non-target species (including humans) or antibodies induced by cross reacting RHDV-like agents. They are:

- *Indirect ELISA (inELISA).* The antigen is linked to the solid phase by a RHDV specific MAb (1H8). It has a slightly higher sensitivity than cELISA, making possible measurement of highly cross-reactive antibodies and it can detect antibodies with low avidity.
- *Solid-phase ELISA (spELISA).* The purified antigen is directly adsorbed to the solid phase and, due to virus deformation, internal epitopes are exposed. Therefore it detects a wider spectrum of antibodies with high sensitivity and low specificity.
- *Sandwich ELISA to detect IgM and IgG in liver or spleen* samples already examined with the virological test. Such a test is particularly useful in those animals which die due to the "chronic" form of the disease when the detection of the virus could be difficult. In this case, a high level of RHDV specific IgM and a low level (if any) of IgG are the unambiguous marker of positivity for RHD.

Experimental data (Capucci and Lavazza, pers. observations; Cooke et al. 2000) showed that there is a firm correlation between the titre in cELISA and the state of protection from the disease, i.e. rabbits with titres equal or higher than 1/10 for antibodies specifically induced by RHDV did not show any sign of disease when challenged with virulent RHDV. In convalescent rabbits, serological titres could be as high as 1:20480, whereas in vaccinated rabbits they are usually comprised between 1:40 and 1:640 according to the time elapsed since vaccination. In young rabbits born from vaccinated healthy does, maternal antibodies usually disappear within 30 days of age but they last longer (45–55 days of age) in young rabbits born from convalescent does, as the passive titres of the young are directly related to those of the mothers. This is true for young rabbits from industrial farms that are weaned quite early (25–35 days of age) whereas in young European rabbits, maternal antibodies can last for 80 days (Cooke et al. 2000). In young rabbits (<35–40 days old), a low level of antibodies (1:80–1:320) could also be induced by an active infection not leading to disease, as commonly occurs in animals of this age. The use of isoELISAs to determine Ig subclasses is therefore essential to correctly interpret serological results. In fact, in the case of passive antibodies, only IgG are found, in vaccinated animals no IgA are detected and in recently infected rabbits, IgM first and then IgA and IgG could be found (Cooke et al. 2000).

Epidemiological Surveillance and Virus Variability

Like all other RNA viruses, RHDV is endowed with considerable genetic variability. This aspect and the rapid diffusion of RHDV throughout the world should also favour a high antigenic variability. In spite of this, since the first identification of RHDV in 1984, all known viral isolates were considered to belong to one serotype. Molecular epidemiological studies did not detect consistent genetic variation up to the end of 1996 and the comparison of the complete sequence of geographically different RHD strains reveals close overall homology, in terms of genome sequence with few or no consequent predicted changes in amino acid composition (differences between 2 and 5%) (Le Gall et al. 1998; Nowotny et al. 1997). Nevertheless, isolates that exhibit temperature-dependant differences in haemagglutinating characteristic were successively described (Capucci et al. 1996a) and then a consistent genetic and antigenic RHDV variant, named RHDVa, was identified at the same time in Italy (Capucci et al. 1998) and Germany (Schirrmaier et al. 1999).

The RHDV Variants (RHDVa and Other Subvariants)

The variant strain RHDVa does not react in an ELISA test with the monoclonal antibody (MAb) 1H8, which is able to protect in vivo experimentally infected rabbits from RHD, and is also less reactive with rabbit sera produced from the original RHDV isolate.

Genetic analysis showed that the amino acid substitutions responsible for the antigenic change are mainly grouped between positions 344 and 370 of the VP60, corresponding to the segment that includes inside the main variable antigenic region of RHDV, similarly to what was described for most members of Caliciviridae family.

Importantly, vaccinated rabbits challenged with an RHDVa variant showed no sign of disease, even if rabbits vaccinated with a low dose are less resistant to the variant than to the original strain of RHDV used as vaccine (Capucci et al. 1998). Very similar data were also reported for the RHDV variant isolated in Germany (Schirrmaier et al. 1999).

As previously mentioned, in order to rapidly distinguish between outbreaks caused by RHDV and RHDVa and to enlarge the capacity to detect new possible variants, an ELISA test based on a panel of MAbs was developed and is commonly applied to typing RHDV strains.

An epidemiological study carried out to compare the rate of diffusion of RHDV and RHDVa in Italy during the last years (Grazioli et al. 2000; Lavazza et al. 2004) has shown that RHDVa is present in most parts of Italy and that it is rapidly replacing the RHDV "classical" strain. The prevalence of each strain during 1997–2004 is reported in Table 1. The highest percentages (70–100%) were found in those regions where the most intensive rabbit production is concentrated (Lombardia, Emilia Romagna, Piemonte, Veneto and Campania) (Fig. 1).

Outside Italy, RHDVa was identified almost contemporaneously in Germany but it also caused the first outbreaks of RHD in the USA during spring 2000 and in Uruguay during winter 2004. It has been detected also in France (2000) and Malta (2004), which suggests that RHDVa could be diffused in other European countries that have been experiencing the disease for many years. Finally, looking at the RHDV genetic sequences deposited at the NCBI databank, it is evident that RHDVa occurs in China as well.

In addition to the data on the presence and diffusion of RHDVa, the epidemiological investigation performed during 2004 by typing the RHDV isolates led to the identification of two viral strains presenting peculiar antigenic characteristics (Capucci, personal observations). In fact, these RHDV strains, isolated during two distinct outbreaks that occurred in widely separated areas and at different times, show different levels of reactivity using the panel of MAbs with respect to both the classical strain and the variant RHDVa. Based on their reactivity with MAbs, these strains

Table 1 Summary of the number of RHDV and RHDVa subtype isolates in Italy during the period 1997–2004

Year	Total RHD cases	RHDV	%	RHDVa	%
1997	211	191	90.5	20	9.5
1998	80	66	82.5	14	17.5
1999	65	39	60.0	26	40.0
2000	134	89	66.4	45	33.6
2001	69	25	36.2	44	63.8
2002	138	61	44.2	77	55.8
2003	63	12	19.0	51	81.0
2004	132	38	28.8	94	71.2

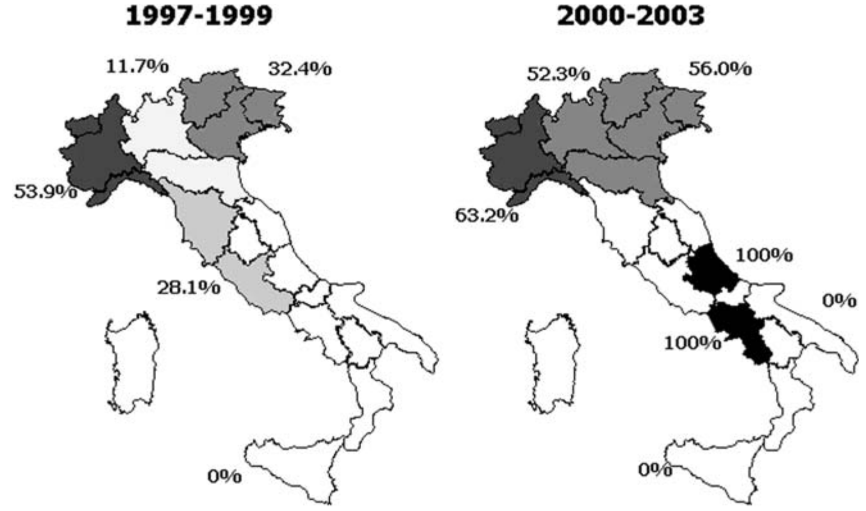

Fig. 1 Prevalence of RHDVa in Italian regions (% of all RHD pos. outbreaks)

could be considered as two further and separate steps of variation of the RHDVa, possibly classified as sub-variants. Indeed the genome sequencing confirmed the existence of some genomic differences in comparison with the RHDV strains previously identified.

The Apathogenic Calicivirus (RCV)

Another virus, rabbit calicivirus (RCV), and related to RHDV, has been identified in healthy rabbits (Capucci et al. 1996b, Capucci et al. 1997). Indeed, preliminary seroepidemiological data had already suggested the existence of one or more 'non-pathogenic' viral strains antigenically related to RHDV. This hypothesis was based on the detection of seropositivity in the sera of laboratory rabbits taken between 1975 and 1985, i.e. 10 years before the occurrence of the disease in Europe, and in farms and colonies where the RHD had never been recorded or vaccination performed (Capucci et al. 1991; Marchandeau et al. 1998a, 1998b; Rodak et al. 1990; Trout et al. 1997). It was confirmed when we observed the spontaneous seroconversion of rabbits reared in the animal facility of our institute in the absence of signs of the disease and mortality, and we then succeeded in reproducing the phenomenon under controlled conditions (Capucci et al. 1995b). RCV is significantly different from the previously characterised RHDV isolates in terms of pathogenicity, viral titre, tissue tropism, and primary sequence of the structural protein: it is avirulent, replicates in the intestine at a low titre, and presents a genomic identity with RHDV of around 92%. However, it is interesting to note that the RCV amino acid sequence of the main capsid protein (VP60) between aa 300 and 311 is the more divergent among all the known sequence of Lagovirus, including two amino acid substitutions and, particularly, three amino acid deletions. This area is known to be highly variable also among different feline calicivirus isolates showing different degree of pathogenicity (Seal 1994). Taken together, these data suggest that the area of the VP60 between aa 300 and 311 could influence the degree of pathogenicity of the caliciviruses.

Experimental infection of hares with RCV failed. In addition, antigenic data and sequence comparisons, demonstrate that RCV is much more closely related to RHDV than to the EBHSV (Capucci et al. 1996b).

The diffusion of RCV inside different commercial rabbitries from different areas in Italy has been evaluated during two separate serological surveys by checking the presence of anti-RHD antibodies in non-vaccinated meat rabbits at slaughter (Capucci et al. 2004). The results of these surveys, the first conducted during 1999 in 39 farms located in northern Italy and the second during 2002–2003 in 21 farms located in central and southern Italy, clearly show that antibodies reactive with RHDV are present in some rabbit populations (Fig. 2). In particular, in almost 30% of controlled farms with no evidence of overt RHD clinical disease, over 80% of animals had titres ≥1/20 (up to 1/1280) for anti-RHDV antibodies by using standard competition ELISA (cELISA). In addition, by using anti-isotype ELISAs, it was clearly shown that from the presence of IgA that an active infection had occurred.

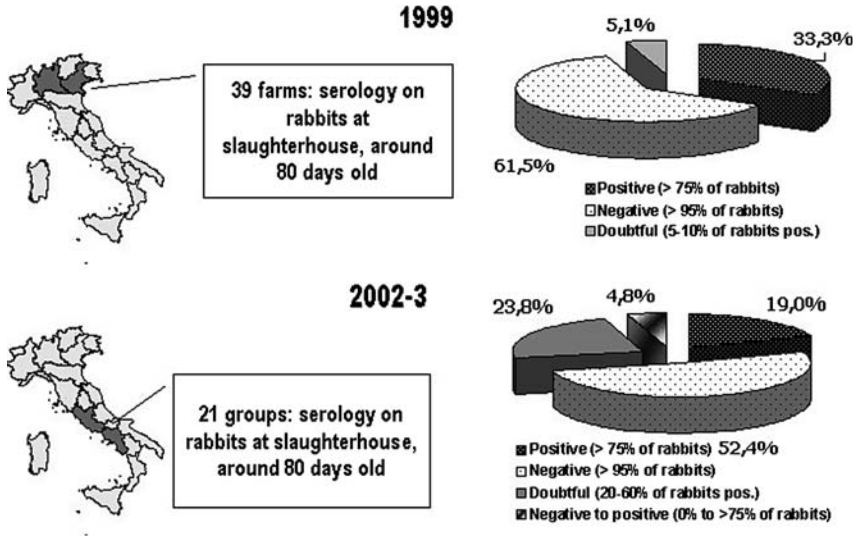

Fig. 2 Results of two serological surveys conducted to verify the diffusion of RCV inside different commercial rabbit rabbitries from different areas of Italy. The criteria for considering the rabbitries as positive, negative, doubtful and negative to positive, are reported in *brackets* in the legend of each pie

The RHDV-Like Viruses

The existence of non-pathogenic caliciviruses was firstly suggested to explain the early discrepancies found in serological surveys of rabbit populations in European countries, Australia and New Zealand.

A study carried out in the UK showed that RNA particles related to RHDV were present in sera collected since 1955, confirming that RHDV-like viruses were present in Europe a long time before the first evidence of RHD (Moss et al. 2002). High antibody levels detected after RHD began to spread through Europe, but in areas where RHD had never been recorded nor suspected, provided further serological evidence that non-pathogenic strains might be present in wild European rabbit populations (Trout et al. 1997; Marchandeau et al. 1998a, 1998b). In addition, some recent data (Marchandeau et al. 2005) provide evidence for the existence of non-protective antibodies raised against a putative RHDV-like virus.

In Australia and New Zealand, several authors (Cooke et al. 2000, 2002; Cooke 2002; Nagesha et al. 2000; O'Keefe et al. 1999) have conducted investigations inside different feral rabbit populations for the presence of non-pathogenic viruses related to RHDV. The serological data obtained on rabbit sera collected before the introduction of RHDV clearly show that antibodies reactive with RHDV were present in feral rabbit populations. Furthermore, the comparison of the results obtained using different ELISA systems (i.e. cELISA vs. IgG-isoELISA; IgELISA vs. spELISA), providing different levels of specificity (Fig. 3), indicated that the great part of these antibodies were cross-reactive antibodies, recognizing antigenic determinants buried inside the structure of the RHDV capsid (Capucci and Horner, unpublished data).

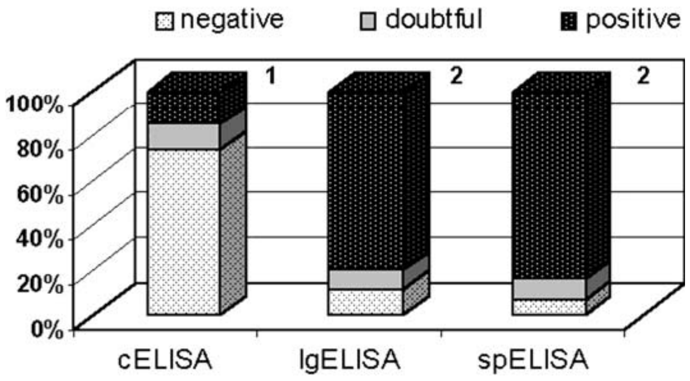

Fig. 3 Comparison of the results obtained using different ELISAs by testing feral rabbit from New Zealand (Dunedin area) collected before the introduction of RHD. (*1*) highest titre 1:20, the majority sera scored 1:10 or less, close to the cut-off of the reaction. (*2*) In all these ELISAs the positive sera scored a large range of values. Around 30% had relatively high titre in both ELISAs

These epitopes could be considered as "common" and possibly "group specific" in all the calicivirus of lagomorphs. Therefore, the serological picture achieved suggested the presence and circulation of infectious agents in the feral rabbit population that induce antibodies partially cross-reactive with RHDV. These putative non-pathogenic RHDV-like viruses are also different from RCV. In fact, the average of serological titres found in Europe by testing with cELISA the sera of animals infected with RCV were 8–16 times higher than the titres found among the feral rabbit sera from New Zealand and Australia positive in IgG isoELISA and spELISA.

In addition, since the antibodies against the putative non-pathogenic RHDV-like viruses could confer a variable level of protection against RHD, the possible role of these non-pathogenic RHDV-like viruses in reducing the impact of RHD has been discussed (Cooke 2002; Cooke et al. 2002; Marchandeau et al. 2005). The main question that arose from the finding of RHDV cross-reactive antibodies within the feral rabbit population was whether these antibodies interfere with RHDV infection and, therefore, with the course of the disease. The data obtained suggest that this is not the case. In fact, there is a firm correlation between the titre in cELISA and the state of protection from the disease: i.e. rabbits with titres in cELISA ≥1/10 show no sign of disease after challenge with RHDV, but only when antibodies are specifically induced by RHDV or RCV. On the contrary, the antibodies directed towards internal determinants (cross-reactive antibodies) have low or no importance from a protective point of view, they are not neutralising and do not interfere with the RHDV infection. Indeed, Cooke et al. (2002) observed that pre-RHD serum antibodies did not protect rabbits from RHD and that, as RHD spread onto previously unaffected sites, heavy mortality occurred even though 70–80% of rabbits had been classified as weakly positive by IgG isoELISA. Similarly, Marchandeau et al. (2005), by studying an outbreak of RHD in feral rabbits in France, observed that some rabbits, despite being seropositive for anti-RHDV antibodies and thus presumably protected, developed a classical acute disease caused by a viral strain that did not differ from the other strains isolated in France during the same period. Therefore, even if the origin of these antibodies remains unknown since they did not isolate the virus that induced them, they concluded that the detected antibodies were probably due to a RHDV-like virus that induces the production of detectable but non-protective antibodies.

Conclusions

The data reported here confirm the *quasispecies* nature of the Lagovirus (RHDV and RHDVa) involved in the aetiology of haemorrhagic diseases of rabbits and stress the importance of continuing epidemiological and antigenic surveillance on the outbreaks of RHD. Indeed, the results show that the combination of serological and virological methods of diagnosis provides novel and highly sensitive means for the identification and characterisation of such viruses, with special regard to genome composition, evolution, features of pathogenicity and molecular epizootiology.

In this context, the potential role of non-pathogenic strains of RHDV-like viruses in RHDV epidemiology should be considered. In particular, it is important that serological surveys use tests that are able to distinguish between antibodies that are protective against RHD and antibodies that are not. To ensure that this is possible, it must be a priority for future research to isolate and characterize RHDV-like strains in order to determine the level of protection that each of them can induce and to better understand the epidemiology of RHD in wild, domestic and industrial rabbit populations.

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Geographic and Seasonal Variation in the Impact of Rabbit Haemorrhagic Disease on European Rabbits, *Oryctolagus cuniculus*, and Rabbit Damage in Australia

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Introduction

Within 12 months of its escape from an island study site in October 1995, rabbit haemorrhagic disease virus (RHDV) spread naturally and rapidly through much of southern Australia (Kovaliski 1998). During the next year, RHDV was deliberately released in European rabbit, *Oryctolagus cuniculus*, populations where rabbit haemorrhagic disease (RHD) had not been detected (Neave 1999). This ensured that few rabbit populations in Australia remained unexposed. Since that time, naturally recurring outbreaks of the disease have been recorded in most rabbit populations studied but the impact on rabbit abundance and rabbit impact has varied greatly. In this chapter, we summarise the epidemiology of RHD in Australia, make some broad generalisations about the impact of the disease on rabbit populations, native fauna and flora and livestock production, illustrate some of the variability in impact using examples from study sites in South Australia and discuss likely underlying causes of that variability.

Materials and Methods

Field studies were conducted in the Flinders Ranges, a hot, semi-arid, inland area (mean annual rainfall approximately 280 mm) and in the Coorong area, a cooler, higher rainfall, coastal area (annual rainfall 450 mm). The primary study sites in the Flinders Ranges were on Gum Creek sheep station (31°25′S, 138°45′E,) and the adjoining section of Flinders Ranges National Park, and in the Coorong district, Coorong National Park and adjoining pastoral grazing properties in the vicinity of Salt Creek (36°08′S, 139°39′E). Numbers of rabbits and native herbivores were monitored by spotlight transect counts and/or dung counts, epidemiology of RHD and myxomatosis was investigated from the antibody status of live-captured/recaptured or shot rabbits (Capucci

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et al. 1997) and vegetation response was monitored in unprotected, fixed vegetation transects and small fenced areas from which herbivores were excluded. Detailed site descriptions and methodology are given in Henzell (1991), Mutze et al. (1998a,b), Cooke et al. (2002a,b) and Mutze et al. (2002). Primary production estimates were from data obtained by interviewing landholders.

Impact of RHD on Rabbit Populations

There was strong regional variation in the initial effect of RHD on rabbit populations, and the disease had its greatest impact in the arid and semi-arid areas of inland Australia (Henzell et al. 2002). These are areas of low productivity used for rangeland sheep and cattle production, aboriginal lands or conservation reserves, where rabbits were formerly uncontrolled and responsible for much, if not most of the total grazing pressure. Since RHD, rabbit numbers have generally declined by 80–95% (Mutze et al. 1998a; Bowen and Read 1998; Neave 1999). The response has been relatively consistent between arid, inland sites and between years within these sites, notwithstanding some recovery recorded where it had the greatest initial impact. At our study site on Flinders Ranges National Park and the adjoining section of Gum Creek sheep station, RHD reduced rabbit numbers to approximately 15% of mean pre-RHD levels during the 6 years after its arrival in late 1995, with no evidence of gradual population recovery (Fig. 1a). At Roxby Downs, an even more arid site 150 km west of the Flinders Ranges (160 mm mean annual rainfall), rabbit numbers recovered gradually from about 1% to reach 17% of mean pre-RHD levels in 2001, declined substantially in 2002 but increased again in late 2003 (Bowen and Read 1998; WMC 2004).

By contrast, the effect of RHD in the more temperate agricultural areas of Australia has been highly variable (Neave 1999). At some sites it has reduced numbers greatly but at others it has had no impact despite regular outbreaks, and there is at least one site where it failed to become established despite several virus releases (Cooke 1999). In general, the disease was least effective in cool, moist areas, in some coastal areas, and during summer in summer rainfall areas (Henzell et al. 2002). The declines in rabbit numbers attributable to RHD range from about 0–80%. There has been considerable variability both between sites and between years within sites. Our study site in the Coorong National Park is a coastal area with about double the effective rainfall of Gum Creek, due not only to its higher rainfall but also to lower evaporation rates. The estimated impact of RHD on rabbit numbers in the Coorong falls roughly in the middle of the range of reductions recorded for temperate areas, with a mean effect of about 36% and estimated annual reductions that varied from 9–73% (Fig. 1b).

As well as reducing rabbit numbers, RHD has also affected the timing and magnitude of seasonal peaks in abundance. In the Mediterranean-like climate

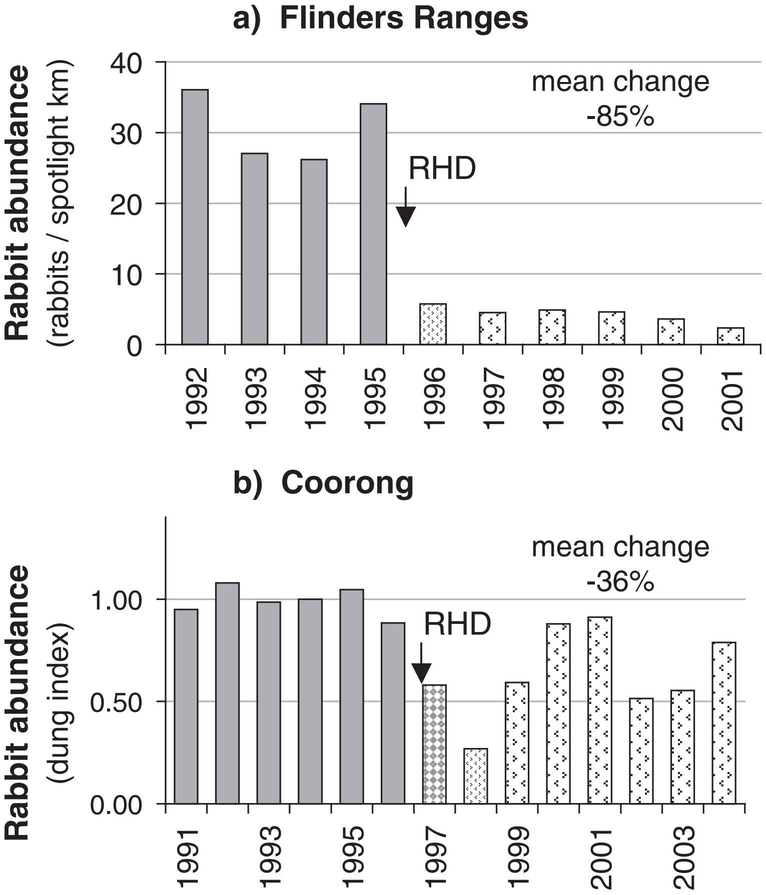


Fig. 1 Changes in mean annual rabbit abundance at two sites in South Australia following the arrival of RHD. Data shown in (**a**) for mean transect counts during the calendar year in the hot, semi-arid Flinders Ranges, and in (**b**) for annual dung weight in the temperate, higher rainfall Coorong area, as a proportion of the expected dung weight from the pre-RHD regression of rabbit dung weight on rainfall (Mutze et al. 2002)

of southern Australia (hot dry summer, cool wet winter), rabbits breed predominantly during winter and spring in response to seasonal pasture growth (Gilbert et al. 1987). Before RHD, peak numbers occurred in late spring before the combined effects of myxomatosis and nutritional stress in summer caused numbers to fall (Fig. 2). Now, because RHD is most active in winter/spring, numbers are greatly reduced during those seasons. Some compensatory survival of late-born young occurs during summer, due to low RHD activity in summer and reduced numbers of early-born juveniles competing for scarce green feed. Consequently, the number of adult rabbits is now highest in late summer, when it was previously lowest. The overall

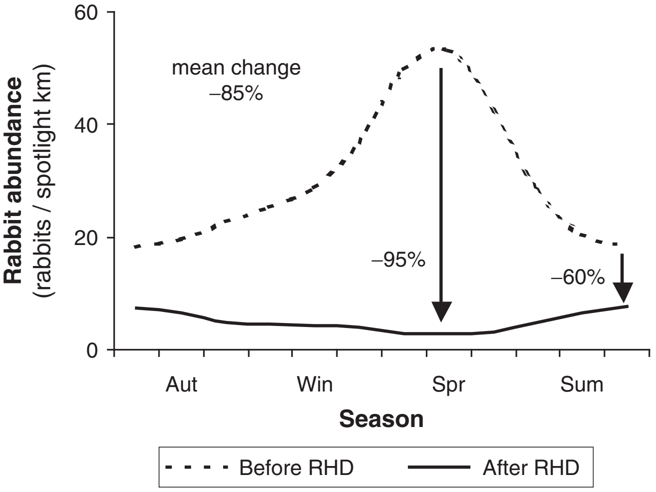


Fig. 2 Seasonal changes in rabbit abundance in the Flinders Ranges, South Australia, before and after the arrival of RHD

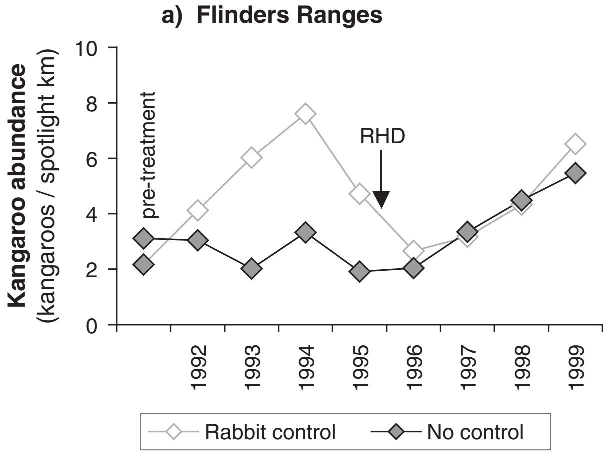
impact of RHD on rabbit abundance is greatest during spring and least during late summer.

In areas of Australia where RHD has had less impact than in the Flinders Ranges site depicted in Fig. 2 the effect may be a flattening of spring peaks rather than complete reversal to a spring trough. In cool climate areas of Australia breeding and population peaks for rabbits are several months later than in the Flinders Ranges but RHD epizootics also tend to occur later and the same patterns apply – RHD can affect both absolute abundance and seasonal variation.

Impact of RHD on Native Fauna

Australian native mammals and birds are not susceptible to RHD (Cooke 2002). Feral cat and fox numbers fell in response to lower rabbit numbers after RHD's escape in 1995 (Sandell and Start 1999), but there is limited information on the effects of these declines in predator numbers on their Australian native prey (Neave 1999; Holden and Mutze 2002).

At Gum Creek, the main mammalian herbivores are rabbits, sheep and kangaroos. We monitored response of Red kangaroos (*Macropus rufus*), Hill wallaroos (*Macropus robustus*) and sheep to rabbit control in four treatment blocks of 3–4 km^2 where rabbits were controlled by warren destruction and compared that response with matching, interspersed rabbit-grazed blocks in the same valley (Fig. 3a). Before RHD, kangaroos responded to rabbit control


a) Flinders Ranges

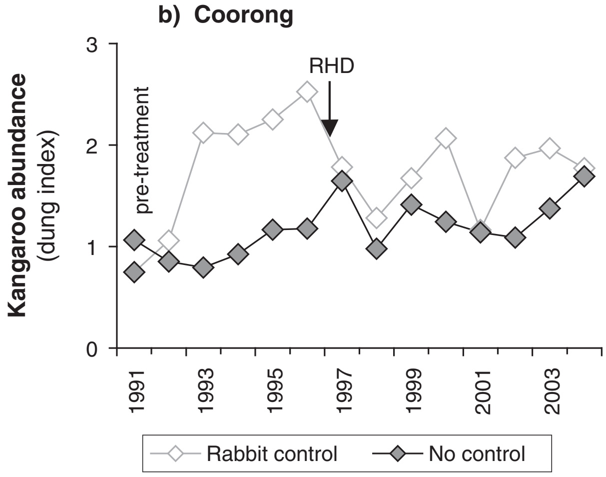

b) Coorong

Fig. 3 Changes in mean annual abundance of kangaroos at two sites in South Australia following the arrival of RHD. Data shown in (**a**) for mean transect counts of red kangaroos (*Macropus rufus*) and euros (*Macropus robustus*) during the calendar year in the hot, semi-arid Flinders Ranges, and in (**b**) for dung weight of western grey kangaroos (*Macropus fuliginosus*) sampled annually in February in the temperate, higher rainfall Coorong area

by moving into areas where rabbit abundance had been greatly reduced to take advantage of the extra food available, and maintained higher numbers there regardless of seasonal conditions. After RHD arrived at the site, there was rapid dispersal to more uniform distribution among treated and untreated plots. As far as kangaroos were concerned, it appeared that RHD had removed rabbits as significant competitors. That appeared to be followed in subsequent years by natural increase on all areas. Sheep flocks tend to learn fixed grazing patterns and consequently responded more slowly to the changes in pasture availability following rabbit control and then RHD, but they too increased their grazing in rabbit-free areas.

In the Coorong National Park, the response of Western grey kangaroos (*Macropus fuliginosus*) was almost identical to that of the kangaroos in the Flinders Ranges (Fig. 3b). Before RHD there was migration into treatments where rabbits were suppressed, followed by rapid dispersal when RHD arrived. Moreover, there was a residual effect of rabbit grazing post-RHD, with kangaroos concentrating on areas where rabbits had been removed especially during those years when RHD was least effective (Figs. 1b, 3b). Although it is debatable whether increasing numbers of the already overabundant large kangaroo species is a conservation gain, their response is quantifiable; many of the smaller marsupial herbivores are now extinct within most of their former range or too rare to measure their response very readily. Coarse-haired wombats (*Vombatus ursinus*) are another large marsupial herbivore found in the Coorong. They too have responded to the changes in rabbit grazing, but more gradually because the native perennial grasses on which they rely took several years to recover after rabbit-grazing pressure was reduced. Post-RHD population recovery has also been reported for arid-zone granivorous rodents, Spinifex hopping mice (*Notomys alexis*) and Plain mice (*Pseudomys australis*), at Roxby Downs (Read 2003) suggesting overall that the benefits of RHD may have been widespread among native mammals, even if poorly quantified for most species.

Impact on Native Flora

Following the reduction of rabbit grazing after RHD, a number of studies recorded general improvement in condition of native vegetation, including the reversal of impending structural changes and/or floristic changes (Sandell and Start 1999). However the changes in timing of seasonal peaks in rabbit abundance since RHD have led to greater benefits for spring-seeding annual plants than for slow-growing long-lived perennial species.

In the vegetation quadrats at Coorong National Park, there are nine species of orchids belonging to six genera (*Caladenia – 4* spp., *Glossodia, Acianthus, Cyrtostylis, Microtis and Pterostylis*), which shoot from underground tubers, produce basal leaves and flower within a few months during winter and early spring before drying off by late spring. Their entire aboveground life-cycle occurs within the seasonal period when RHD has reduced rabbit numbers most. We monitored vegetation recovery after RHD arrived in 1996 in areas cleared of rabbits and compared that with recovery in rabbit-grazed areas. We do not know precisely how many orchids were present in 1996 because vegetation surveys were originally conducted in November (late spring) and we did not record them. Nevertheless, there was a lag in the first few years after RHD spread until the orchid population

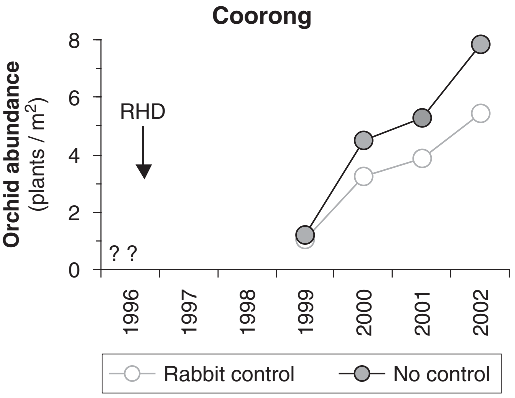


Fig. 4 Changes in abundance of orchids at the Coorong National Park in South Australia following the arrival of RHD

Table 1 Change in the number of moderately palatable perennial plants in vegetation transects following selective rabbit control in the Flinders Ranges, South Australia

	Rabbit grazed (plants/transect/year)	No rabbits (plants/transect/year)
Before RHD (1992–1995)	−1.6	+4.2
After RHD (1995–1997)	+5.2	+4.2

began to increase rapidly (Fig. 4), possibly a period of exponential increase from a very low base, possibly because they did not occur in most quadrats and seed needed to arrive from some other source, and/or possibly because other changes had to occur first, as was the case with the wombats. Mid-September orchid counts began in 1999, and in the following 4 years mean density of orchids increased six-fold, the number of quadrats containing orchids increased three-fold and the number of species detected inside quadrats increased from 5 to 9. In this case, RHD reduced rabbit grazing to negligible impact in winter-spring despite a mean reduction in rabbit numbers that was generally less than 40%.

At Gum Creek, we also monitored recovery of native vegetation in the areas cleared of rabbits and compared that with recovery in rabbit grazed areas. Most of the highly-palatable perennial shrubs were removed from this study area by livestock over-grazing in the late 1800s and were replaced by exotic weeds and, to some extent, by increased abundance of a range of endemic shrubs that are only moderately palatable to rabbits, native herbivores and livestock. The moderately-palatable shrubs tend to increase in abundance during years of above average rainfall and decline in dry years. In the 3 1/2 years before RHD, net shrub recruitment occurred only in areas where rabbits had been removed by warren destruction (Table 1) and the proportion of transects where shrubs increased differed significantly between treatments (Mutze et al. 1998b). After RHD, shrubs increased equally in rabbit-grazed and rabbit-free areas despite barely average rainfall.

However, not all news for vegetation recovery post-RHD is good, particularly for the more palatable perennial plants. Rabbit grazing on seedlings is most severe in late summer when there is little alternative green feed available, and that is when RHD has provided least benefit. The plight of these species is illustrated by that of mulga (*Acacia aneura*), the dominant small tree across vast tracts of central Australia. A study of mulga recruitment in the northern Flinders Ranges has recorded almost no seedling survival outside of rabbit-proof fenced areas during the past 28 years. Even at two sites where RHD reduced rabbit population density to approximately one rabbit/km^2, rabbits still removed up to half the seedlings in a few months. Since mulgas grow slowly, they remain susceptible to rabbit grazing for many years, and recruitment is prevented. This is just one example of a widespread problem. Many other arid zone trees and shrubs have highly palatable seedlings and their long-term outlook remains dire despite the substantial reduction in rabbit numbers (Denham and Auld 2004). In higher rainfall areas similar problems occur but are less uniform: in the Coorong National Park, rabbits still prevent regeneration of drooping she-oaks (*Allocasuarina verticillata*) even though good recruitment has been recorded post-RHD on the nearby Narrung Peninsula. Across Australia, many palatable trees and shrubs are gradually dying out due to rabbit grazing. This poses a threat to entire vegetation communities and their dependent fauna, not just to individual species.

Impact of RHD on Livestock Production

The livestock industry of inland Australia is based on grazing of native pastures and in the past rabbits were major competitors for feed. The rabbit-prone area used for cattle grazing covers approximately 600,000 km^2. Estimates of improved productivity since RHD equate to increased net production of approximately \$43/km^2/year, or \$A206 million in 8 years. The increased production has in many areas been accompanied by recovery of native vegetation; that is, cattle are eating some but not all of the extra food available as the result of reduced rabbit numbers. Recent estimates of increased sheep production in higher rainfall areas since RHD (Vere et al. 2004) indicate total benefits of \$A7 million–\$A38 million per year, the same order of magnitude as those for arid zone cattle production, and additional benefits to arid zone sheep production are likely to be comparable but are not quantified. Landholders in agricultural areas also have benefited from reduced rabbit control costs (Saunders et al. 2002).

Why has RHD had Less Impact in the Higher Rainfall Zones of Australia?

In Australia, the impact of RHD is frequently reported to be patchy on a small geographic scale, e.g. killing rabbits in one locality without affecting another group of warrens nearby. The more interesting phenomenon, although perhaps not unrelated, is that RHD has had far greater impact on rabbits in arid and semi-arid inland areas than it has on rabbit populations in the relatively cool, moist agricultural areas of southern and eastern Australia. The following are possible contributory factors:

Seasonal Timing of RHD Outbreaks Allows High Survival

Young rabbits are more likely to survive infection with RHD than susceptible sub-adult or adult rabbits (Robinson et al. 2002b). Therefore, survival rates may be higher and subsequent population recovery more rapid if epizootics pass through rabbit populations during the breeding season than if they pass through when no kittens are present. Epidemiological studies in both Europe (Calvete and Estrada 2000) and Australia (Cooke 2002) indicate that survival of these young rabbits plays a critical role in enabling rabbit populations to withstand RHD.

In the Australian arid zone, RHD epizootics generally occur in winter soon after the commencement of breeding, before any kittens are present. Such epizootics cause high mortality amongst susceptible young adult rabbits born late in the previous year (Mutze et al. 2002) and leave a breeding population consisting almost entirely of older does with high antibody titres. RHD continues to trickle through the population until the end of spring but, outside of the major epizootics, kittens tend not to become infected until they lose maternal antibody protection between 5–11 weeks of age (Cooke et al. 2000), at which time infection is usually lethal. If the annual disease epizootic occurs 1–2 months after commencement of breeding, the susceptible does die of RHD and their unweaned offspring die of malnutrition. However, in the minority of epizootics that occur >2 months after commencement of breeding, annual recruitment is higher because there are young rabbits of 4–8 weeks of age in the population that have a high probability of infection and recovery.

In cooler high rainfall areas, by contrast, RHD epizootics most commonly occur at the height of the breeding season, during spring, when kittens are most abundant. Surviving kittens sero-convert, reach adult size within a month or two and add to the RHD-immune breeding population in future years. Rabbits first infected as adults suffer much higher mortality from RHD (R. Sinclair, unpublished data).

Nevertheless, some evidence relating to the importance of seasonal timing of RHD for juvenile recruitment is contradictory. Henzell et al. (2002) analysed population changes at 78 sites throughout Australia during the initial spread of RHD. They found that differences in the effectiveness of RHD along the climatic gradient between hot, dry areas and cool, moist areas were independent of season. That result indicates that survival of young rabbits did not contribute significantly to the lower effectiveness in cool, moist areas—if it had, a marked interaction between season and survival rates would have been expected, to reflect the seasonality of breeding. Furthermore, they noted that the equal proportions of young rabbits found at the Coorong and Flinders Ranges sites (Mutze et al. 2002) suggest that juvenile recruitment rates do not differ, at least between these sites.

Another Related Calicivirus Protects Against RHD

It is likely that a non-pathogenic, RHD-like virus is reducing the effectiveness of RHD in some wetter parts of Australia (Cooke et al. 2002a). The evidence for the presence of such a virus is indirect, and is based on the fact that some rabbits show unusual antibody profiles, reacting strongly to only one of the four ELISA tests for RHD antibodies (Capucci et al. 1997) (Table 2). The putative RHD-like virus is therefore antigenically distinct, and presumably genetically distinct, from RHDV. Its antigenicity differs from the non-pathogenic rabbit calicivirus (RCV) described from Italian rabbit farms (Capucci et al. 1997) which shares a common serological profile with RHDV and completely protects rabbits from RHDV challenge.

Antibody profiles indicating the presence of a putative RHD-like virus were common in rabbit sera collected from many parts of Australia before RHD was introduced (Robinson et al. 2002a, Table 2). However, today they are mainly seen in higher rainfall areas and it is postulated that RHD is most likely to be impeded by interaction with the putative virus where annual rainfall exceeds 500 mm (Cooke et al. 2002a). Mortality caused by RHD during its initial spread was much lower in those areas and Henzell et al. (2002) considered immunity provided by previous infections with an RHD-like non-pathogenic virus to be the most likely explanation. During recent experimental field

Table 2 Response to RHD ELISAs at 1:40 dilution or greater in rabbit sera from the Flinders Ranges, South Australia. Mean titre for IgG is taken from only those samples that were positive at ≥1:40 dilution

	cELISA positive	IgG positive	IgG mean titre	IgA positive	IgM positive
Before RHD (*n* = 44)	nil	23%	1:150	nil	nil
After RHD (*n* = 1056)	37%	44%	1:900	18%	9%

releases of RHDV on bait at Belair, South Australia (>800 mm rainfall), we found survival rates of European rabbits with these anomalous antibody profiles to be intermediate between that of rabbits with no antibodies and those with typical RHD antibody profiles (G. Mutze and R. Sinclair, unpublished data). Such differences in mortality would be sufficient to greatly reduce the impact of RHD at a population level.

Similar antibody responses and RHDV-like virus particles also have been recorded in archival sera from various parts of Europe and New Zealand. However, attempts to distinguish between the genetic sequence of RHDV and sequences of viruses recovered in archival samples, or those of circulating non-pathogenic field viruses (except for RCV in Italy), have so far been unsuccessful in the United Kingdom (White et al. 2004), in Australia and in New Zealand. On that basis, White et al. (2002, 2004) have argued that there may be only one calicivirus in rabbits in the United Kingdom: i.e. that RHDV may cause either chronic infection (non-lethal, producing "non-pathogenic" antibody response) or acute disease (lethal, or producing "RHD" antibody response in survivors). Until such time as genetically distinct non-pathogenic forms of the virus are isolated, that possibility remains open. It is, however, difficult to suggest a plausible explanation of why chronic RHDV was present in many rabbit populations for decades without any evidence of acute disease, yet suddenly switched to acute transmission at the time that another (genetically similar) batch of the virus was introduced and spread through the country.

Possible Negative Interactions with Myxomatosis

RHD has changed the seasonal timing of myxomatosis outbreaks in many areas (Mutze et al. 2002, Bruce et al. 2004). The change has been most pronounced in semi-arid areas, where RHD epizootics occur predominantly in winter and myxomatosis has shifted from predominantly spring/summer epizootics to autumn epizootics. That change does not appear to have greatly affected the impact of myxomatosis on rabbit populations: evidence from Gum Creek indicates that rabbits susceptible to myxomatosis still have 50% lower survival probability in the year after their birth than those that have acquired immunity to the disease.

In agricultural areas, although myxomatosis now tends to occur slightly later than previously (Mutze et al. 2002), both diseases still occur predominantly in spring and the potential for negative interactions seems greater. We have recently sampled several rabbit populations in higher rainfall areas with very low sero-prevalence of myxoma antibodies throughout winter and spring. These may have been chance observations made shortly before myxomatosis outbreaks. However, an alternative hypothesis could be that suitable vectors for transmission of RHDV and myxoma are generally abundant only in spring. The timing of myxomatosis outbreaks in the agricultural areas of southern Australia is clearly linked to seasonal abundance of European rabbit fleas (most common in late winter-spring) and mosquitoes (spring-early summer) (Fenner and Fantini 1999). These insects can also transmit RHDV, as can bushflies and blowflies that also increase in abundance in spring, although the relative importance of different vectors in promoting RHD outbreaks remains unclear (Cooke and Fenner 2002). If both viruses compete for young adult rabbit hosts during spring, RHD-related mortality may prevent myxomatosis from being transmitted effectively (or vice-versa) by reducing the density of susceptible rabbits at a critical time, without greatly changing the overall level of annual mortality. Because of the lack of insect vectors in extremely arid areas, myxomatosis had little impact before RHD so could not be greatly affected by RHD.

Developing a better understanding of the interactions between RHD, myxomatosis and non-pathogenic caliciviruses appears to be the greatest challenge to further understanding the epidemiology of RHD in Australia's European rabbits.

Rabbit populations in Australia began to recover from the impact of myxomatosis within a few years of its introduction, due to coevolution of genetic resistance in rabbits and less virulent field strains of myxoma virus (Fenner and Fantini 1999). No widespread rabbit population recovery, significant genetic resistance in rabbits, or emergence of less virulent strains of RHDV or myxoma virus have yet been reported following the introduction of RHDV in any country. Comparisons of future developments in New Zealand, where myxoma is not present, with those in Europe and Australia, may help to clarify the role of interactions between the two viruses.

Poor Persistence and Transmission of RHD Virus

There is little evidence to indicate how and where RHD persists between outbreaks. If outbreaks are most commonly initiated by virus shed by chronically-infected "carrier" rabbits, persistence of virus is likely to be similar in all regions. White et al. (2004) conclude persistent infection to be the most likely explanation of epidemiological patterns observed in the United Kingdom, although there is no experimental evidence that chronically infected rabbits shed virus that can then cause acute RHD infection. Alternatively, if outbreaks are usually initiated from external virus sources (e.g. in soil or old rabbit carcases in deep warrens), virus persistence could vary according to environmental conditions and contribute to the variable impact of RHD. Henzell et al. (2002) suggested that to be the most likely explanation for the reduced effectiveness of RHD during summer in summer rainfall areas (in the second climatic principal gradient in their analysis; they considered persistence or transmission unlikely to have influenced effectiveness of RHD along the first, hot-dry to cool-moist, climatic gradient). RHDV injected into bovine liver samples was recently shown to persist in the environment at ambient temperatures for at least 3 months (Henning et al. 2005) which suggests that carcases of rabbits could provide a persistent reservoir of virus under some circumstances.

Poor RHD transmission can sometimes limit disease outcomes on a small geographic scale, as indicated by reports of patchy outbreaks of RHD in agricultural areas. There is also some indication of regional differences in the rates of transmission of the disease: rabbits from the Coorong are more likely to reach 12 months age without being infected than rabbits at Gum Creek, despite annual RHD epizootics in both places (Mutze et al. 2002). However, it is not clear whether regional variation in either persistence or transmission of RHDV is strong enough to generate regional differences in impact.

Higher Productivity Allows Rabbit Populations to Recover from Losses to RHD

Reproductive output of rabbits in areas with temperate climates and reliable winter rainfall is higher than it is in the arid inland areas subject to high temperatures and unreliable, low winter rainfall (Gilbert et al. 1987). Consequently, individuals dying as a result of annual RHD epizootics may be more readily replaced in higher rainfall areas. This possibility was considered by Henzell et al. (2002) who analysed the population changes associated with RHD outbreaks occurring in different seasons. They considered it unlikely that high reproductive output was masking the impact of RHD because (a) the seasonality of rabbit breeding should have introduced a seasonal component into the effectiveness of RHD, for which there was no statistically significant evidence and (b) it should also result in rabbit populations in high rainfall areas having a higher proportion of juveniles, but this also appeared not to be the case. Direct evidence to indicate whether high reproductive output is important for maintaining rabbit populations in high rainfall areas is lacking. Although productivity is the simplest explanation of regional differences in RHD impact, it is perhaps the least likely: the ability of rabbit populations to regain numbers appears to be very sensitive to factors other than the number of juveniles produced.

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Conservation and Management

Conservation of Endangered Lagomorphs

Andrew T. Smith

Introduction

There is a widely held perception that all lagomorphs are fecund ("they breed like rabbits") and are sufficiently generalist in their ecology such that they can be considered amply buffered from environmental change and thus be unlikely candidates to appear on lists of threatened species. It is true that many lagomorph species fit this perception; indeed some of the most important game animals in the world are lagomorphs, such as the European rabbit and many cottontail and hare species. However, the generalization is untrue for a surprising number of lagomorphs. Approximately a quarter of all lagomorphs are threatened with extinction (nearly the same percentage as for all mammal species; Baillie et al. 2004). In this chapter I will review the historical treatment of threatened lagomorphs, outline the current threatened (global Red List status) of lagomorphs, and highlight ongoing proactive conservation measures being taken to ensure that we can preserve the important and rich diversity of lagomorphs.

The Early Years

It was not always understood that lagomorphs were so susceptible to environmental threats. The 1972 and 1978 IUCN Red Data Books (Goodwin and Holloway 1972, 1978) listed only four lagomorph species (*Caprolagus hispidus, Nesolagus netscheri, Pentalagus furnessi, Romerolagus diazi*; Table 1). Indeed, these four species were the first to gather attention among lagomorph conservationists. Much of this early awareness was due to biologists from the United States or the United Kingdom working abroad, such as Jeff McNeely for *Nesolagus*, and Diana Bell, John Fa and William Oliver who pioneered work in India on *Caprolagus* and in Mexico on *Romerolagus. Caprolagus* and *Romerolagus* were even afforded Appendix I protection under CITES, although they were not then nor are they now threatened with

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Table 1 Chronological accounting of the threatened status of lagomorphs as reflected in IUCN Red Lists of Threatened Animals. For years 1994 and earlier: Ex = Extinct; E = Endangered; V = Vulnerable, R = Rare; I = Indeterminate; K = Insufficiently Known. For 1996 and 2004: EX = Extinct; CR = Critically Endangered; EN = Endangered; VU = Vulnerable; LR(nt) = Lower Risk, Near Threatened (NT = Near Threatened became an independent category in 2001 with the advent of Version 3.1 of the IUCN Red List Categories; IUCN, 2001); DD = Data Deficient. The listing of species reflects the current taxonomic standard for lagomorphs (Hoffmann & Smith, 2005); some of the listings of subspecies in earlier Red Lists are presented as full species below

	1972/ 1978[a]	1988[b]	1990[c]	1994[d]	1996[e]	2004[f]
Leporidae						
Brachylagus idahoensis				V	LR(nt)	LR(nt)
Bunolagus monticularis		E	E	E	EN	CR
Caprolagus hispidus	E	E	E	E	EN	EN
Lepus callotis				I	LR(nt)	LR(nt)
Lepus castroviejoi					VU	VU
Lepus fagani					DD	DD
Lepus flavigularis		E	E	E	EN	EN
Lepus hainanus				K	VU	VU
Lepus insularis				R	LR(nt)	LR(nt)
Lepus othus				K		
Lepus yarkandensis					LR(nt)	LR(nt)
Nesolagus netscheri	R	I	I	E	CR	CR
Nesolagus timminsi						DD
Pentalagus furnessi	E	E	E	E	EN	EN
Romerolagus diazi	E	E	E	E	EN	EN
Sylvilagus cunicularius					LR(nt)	LR(nt)
Sylvilagus dicei				K	EN	EN
Sylvilagus graysoni			E	E	EN	EN
Sylvilagus insonus			Ex	E	CR	CR
Sylvilagus mansuetus				I	LR(nt)	LR(nt)
Sylvilagus palustris hefneri			E	E	EN	EN
Sylvilagus transitionalis					VU	VU
Ochotonidae						
Ochotona alpina nitida				V		
Ochotona argentata (= O. helanshanensis = O. alpina argentata)				V	CR	CR

(*Continued*)

Table 1—Continued

	1972/ 1978[a]	1988[b]	1990[c]	1994[d]	1996[e]	2004[f]
Ochotona cansus morosa				V	DD	DD
Ochotona cansus sorella				E	EN	EN
Ochotona forresti				K	LR(nt)	LR(nt)
Ochotona gaoligongensis				I	DD	DD
Ochotona hoffmanni (= O. alpina hoffmanni = O. alpina sp.)				V	VU	VU
Ochotona huangensis (= O. thibetana huangensis)				I	EN	EN
Ochotona iliensis				I	VU	VU
Ochotona koslowi			V	V	EN	EN
Ochotona muliensis			R	I	DD	DD
Ochotona nubrica				I		
Ochotona pallasi hamica				E	CR	CR
Ochotona pallasi sunidica				I	EN	EN
Ochotona princeps goldmani					VU	VU
Ochotona princeps lasalensis					VU	VU
Ochotona princeps nevadensis					VU	VU
Ochotona princeps nigrescens					VU	VU
Ochotona princeps obscura					VU	VU
Ochotona princeps sheltoni					VU	VU
Ochotona princeps tutelata					VU	VU
Ochotona pusilla				V	VU	VU
Ochotona thibetana sikimaria				E	CR	CR
Ochotona thomasi				I	LR(nt)	LR(nt)
Prolagus sardus			Ex	Ex	EX	EX

[a]Goodwin & Holloway (1972; 1978); [b]IUCN Conservation Monitoring Centre (1988); [c]IUCN (1990); [d]Goombridge (1993); [e]Baillie & Groombridge (1996); [f]IUCN (2004)

extinction due to international trade (early listing of these species in CITES simply reflects the growing concern at that time for their protection along with an incomplete understanding of the nuances of CITES listings). At the time of the first World Lagomorph Conference in 1979 (Myers and MacInnes 1981) conservation of lagomorphs was still poorly understood (see their Table 1, page xvii-xix). Conservation papers were presented on six species: *Bunolagus monticularis* (Robinson 1981); *C. hispidus* (Ghose 1981); *N. netscheri* (McNeely 1981); *P. furnessi* (Hayashi 1981); *R. diazi* (López-Forment and Cervantes 1981; Granados 1981); and *Sylvilagus transitionalis* (Chapman and Stauffer 1981).

By the late 1980s (IUCN Conservation Monitoring Centre 1988), six lagomorph species were listed as threatened: *Lepus flavigularis* in Mexico and *B. monticularis* in South Africa in addition to the four listed in 1978 (Table 1). This listing was greatly expanded in the 1990 IUCN Red List of Threatened Animals (IUCN 1990) to include two pikas (*Ochotona koslowi*, *Ochotona muliensis*) and nine leporids (*B. monticularis, C. hispidus, L. flavigularis, N. netscheri, P. furnessi, Romerolagus diazi, Sylvilagus graysoni, Sylvilagus insonus, Sylvilagus palustris hefneri*; Table 1). At the same time the Lagomorph Action Plan (Chapman and Flux 1990) was produced which provided the first comprehensive treatment of the conservation status of the lagomorphs (Chapman et al. 1990). In total six pikas, two hares and nine rabbits were considered, although the status of several of these was indeterminate. Most important, each genus of lagomorph was treated in detail in dedicated chapters, which provided the most comprehensive coverage of lagomorph biology and systematics to date.

The next major step forward in lagomorph conservation was the compilation of threat status for the 1994 IUCN Red List of Threatened Animals (Groombridge 1993). This effort was marked by two factors: it was the first attempt to evaluate all species of lagomorph, and it was the last time that the IUCN qualitative criteria were used for Red Listing. At this time—before modern advances in communications—I attempted to assemble the most current information available on all lagomorph species through the mail (often with multi-month lag times between communications). I then made judgment calls on whether the data on each species best supported the designations of Endangered, Vulnerable or Rare. Overall, 12 species of lagomorph were listed as threatened (eight Endangered [E]; three Vulnerable [V]; and one Rare [R]); while an additional seven species were considered Indeterminate (I) and four Insufficiently known (K; Table 1).

Quantitative Red-Listing

A major breakthrough occurred in 1994 with the advent of quantitative criteria for assessing the Red List status of species (IUCN 1994). The 1996 Red List of Threatened Lagomorphs (Baillie and Groombridge 1996; Table 1) used these criteria for the first time. I used available data from the 1994 Red List process as well as newly submitted information to formulate these listings; some species were downlisted while many at the species and subspecies level were added. The status of many species changed between the two listing processes. Five ochotonids and 12 leporids were listed as threatened at the species level, and numerous threatened subspecies were identified. Several species were identified as Data Deficient.

Subsequent review of the Red Listing process has resulted in minor, yet important, modifications in the quantitative Red List criteria (IUCN 2001) that are now applied to the listing process. In addition, the SSC (Species Survival Commission) and its Red List Program have formalized the listing process; each threatened species proposal is made with the assistance of one or more assessors, and this assessment is reviewed by two evaluators. Changes in listing status must be made formally through the Red List Program. As it has been nearly a decade since the Red List status of most lagomorphs has been reviewed, the process of re-evaluation is currently underway and will undoubtedly lead to changes in the current status as reflected in the 2004 Red List. The first re-evaluation of a lagomorph using this process resulted in a change of the status of *B. monticularis* from Endangered to Critically Endangered (see below).

Endangered Lagomorphs

This section highlights what is known about lagomorph species and subspecies that have been determined threatened or near-threatened with extinction. The base-line for this list is the current IUCN Red List; also considered are species that do not presently have threatened status, but have been identified during the ongoing construction of the Lagomorph section of the SSC Global Mammal Assessment (IUCN 2005). While the list is long, it remains incomplete. Within several of the more widely-distributed species, there are probably threatened isolated subspecies that have yet to receive conservation attention.

Leporidae

Pygmy Rabbit (*Brachylagus idahoensis*) - LR(nt). The Pygmy rabbit is an interesting case. Distributed across the high big sagebrush habitat in western United States, it has an isolated population in the Columbia Basin of Washington State. This population has not been afforded subspecies status, even though it appears to be genetically distinct from other Pygmy rabbits. Six small populations were known in the 1990s, but by 2004 these had all disappeared (Hays 2004). The Columbia Basin population was listed in the

US Endangered Species Act as an emergency designation in 2001—made permanent in March 2003. Active efforts, including captive breeding, are in place to restore this population; it is estimated that only 6–10% of its suitable habitat remains. Throughout the range of the species the Great Basin sage habitat is under threat, and it is thought that Pygmy rabbits correspondingly may be on the decline. In some areas, however, they seem to be healthy. This situation is being carefully monitored, and the species appears to be a candidate for a Vulnerable Red List classification.

Riverine Rabbit (*Bunolagus monticularis*) - CR. The Critically Endangered Riverine rabbit lives as a riparian habitat specialist only in the Central Karoo region of South Africa; it has declined about 60% in the past 20 years (Ahlmann et al. 2000; Friedman and Daly 2004). The species occupies only fragmented private land, and all efforts underway to conserve it entail working with local landowners in conservancies. Currently the Riverine rabbit Working Group, led by Victoria Ahlmann, is engaging in surveys, monitoring, outreach and education (Ahlmann and Collins 2004). Matthee and Robinson (2004) suggest that preservation of suitable habitat should receive the highest priority for restoration of the Riverine rabbit, and that breeding programs, translocations and reintroductions are inappropriate at this time.

Hispid Hare (*Caprolagus hispidus*) - EN. The Endangered Hispid hare occupies tracts of early successional grasslands along the foothill regions of the Himalayas, from Uttar Pradesh through Nepal and West Bengal to Assam. This species was once believed to be extinct, until capture of a specimen in 1971 brought renewed hope for the species. While it has been located in a number of wildlife sanctuaries, it has disappeared from several of these and its specialist habitat type is heavily impacted by human activities (Bell et al. 1990; Maheswaran 2002).

White-sided Jackrabbit (*Lepus callotis*) - LR(nt). This species, found from southern Mexico north to New Mexico, USA, has previously been listed as Near Threatened (Chapman et al. 1990; Flux and Angermann 1990); however, a recent analysis by The Mexican Association for the Study and Conservation of Lagomorphs (AMCELA) has determined that it is of Least Concern. It is, however, listed as state Threatened in New Mexico, USA (New Mexico Game & Fish 2002).

Broom Hare (*Lepus castroviejoi*) - VU. The Broom hare is IUCN Vulnerable based on its restricted geographic range in northern Spain. Apparently its population, however, is stable within this range (Alves and Niethammer 2003), and this listing needs to be updated with a current Red List Assessment.

Corsican Hare (*Lepus corsicanus*) - NE. The Corsican hare (or Apennine hare) is not currently Red Listed; a preliminary assessment of Endangered has been proposed. This species occurs throughout central and southern Italy and on Sicily; it has been introduced in historical times to Corsica. The provisional Endangered classification refers to the Italian peninsula range of the species, where it occurs at low density in fragmented habitat. Populations are decreasing due to habitat degradation, hunting pressure, and competition with *Lepus europaeus*. In addition, the Corsican hare is highly susceptible to EBHS. Populations on Sicily appear to be stable, and the population status on Corsica is unknown; the species is legally hunted on these two islands (Angelici and Luiselli 2001).

European Hare (*Lepus europaeus*) - LR(lc). Not currently Red Listed, the European hare presents an interesting case of a species which is widespread and commonly hunted, yet has suffered demonstrable declines across its range over the past four decades (Ballesteros et al. 1996; Slamečka et al. 1997; Schmidt et al. 2004; Smith et al. 2005). Upon close inspection the European hare may meet the decline criteria for listing as Vulnerable, and a comprehensive Red List Assessment is badly needed. A combination (perhaps with different weights across the range of the species) of habitat loss due to agricultural intensification, lowered fertility due to agricultural practices, predators, and/or EBHS may be causative agents of the widespread decline of the European hare.

Ethiopian Hare (*Lepus fagani*) - DD. The Ethiopian hare, found on high plateaus in western Ethiopia, is Data Deficient, and a thorough Red List Assessment of this species is needed.

Tehuantepec Jackrabbit (*Lepus flavigularis*) - EN. The Tehuantepec jackrabbit is currently Red-listed as Endangered. The area of occupancy may be as little as 67 km^2 in coastal Oaxaca, Mexico, comprised of three separated and small populations. The total population is estimated as fewer than 1,000 individuals, and these numbers continue to decline. The Tehuantepec jackrabbit is jeopardized by habitat alteration and degradation by invasive alien grasses, human-induced fires, encroaching agriculture, cattle-raising activities, predation, and hunting. Although recognized as critically endangered by Mexican law, lack of enforcement further puts the hares at risk (Farías 2004, Farías et al. 2008, this book).

Hainan Hare (*Lepus hainanus*) - VU. Endemic to Hainan Island, this hare is Red-listed as Vulnerable. Ninety percent of its original dry savannah habitat has been destroyed. Artificially cleared deer ranches currently provide most of its extant habitat. It has been estimated that no more than 2 km^2 of optimal habitat remains, with a population of no more than 250–500 animals. Hainan hares are also poached, in spite of their classification as a First-class protected species under Chinese law. Additionally, the species may face competition from feral European rabbits (Lazell et al. 1995).

Black Jackrabbit (*Lepus insularis*) - LR(nt). The Black jackrabbit is Red-listed as Near Threatened, although it occupies a restricted geographic range of less than 100 km^2 on Espíritu Santo Island in the Gulf of California, Mexico. The hare is apparently common on the island, but it remains at risk from introduced predators and competitors, and disturbance by humans. Its situation deserves to be monitored closely (Cervantes et al. 1996).

Alaskan Hare (*Lepus othus*) - LR(lc). This species, found on the northern reaches of Alaska (USA), is poorly known, and its status needs to be assessed. In 1994 its Red List status was Insufficiently Known.

Mountain Hare (*Lepus timidus*) - LR(lc). While a common and widespread species, some subspecies or populations (such as in northern Ireland and the

Alps) may be threatened at present. The Irish hare (*L. t. hibernicus*) has undergone a steep decline in population density and is in need of additional study and a Red List Assessment (Dingerkus and Montgomery 2002).

Yarkand Hare (*Lepus yarkandensis*) - LR(nt). The Yarkand hare is currently assigned as Near Threatened, but there are compelling reasons why this species should be carefully monitored and reassessed. It occupies only the isolated oases that punctuate the desolate Taklimakan Desert of western China. These sites are increasingly being developed. Thus the species is at risk of localized inbreeding within oases, habitat loss, and potential overharvest; currently there is significant genetic subdivision and low gene flow among isolated populations (Gao 1983; Li et al. 2005).

Sumatran Rabbit (*Nesolagus netscheri*) - CR. The Critically Endangered Sumatran rabbit is one of the rarest of lagomorphs. It is known from only about a dozen museum specimens (collected between 1880–1916), and it has been seen only occasionally since that time. FFI (Fauna and Flora International) published a picture in 1998 of a Sumatran rabbit captured in a camera trap, one of two individuals they identified in this manner. Apparently this has always been a rare rabbit occupying dense forest at moderate elevations on Sumatra, habitat that is under increasing development pressure (Flux 1990). We clearly need more information on this species.

Annamite Striped Rabbit (*Nesolagus timminsi*) - DD. The Annamite Striped rabbit, first "discovered" by scientists in 1995 and subsequently described by Surridge et al. (1999) and Averianov et al. (2000) remains poorly known. It is currently Red-listed as Data Deficient, although all available evidence points to a Near Threatened or Threatened category. Locally the species is heavily hunted (largely with snares). It has been found in aseasonal wet forests that generally occur on the seaward facing slopes of the Annamite mountains, although its extent of occurrence remains unknown. Several records come from degraded forest areas, thus it may demonstrate flexibility in its habitat preferences. Further study of this species is needed.

European Rabbit (*Oryctolagus cuniculus*) - LR(lc). It may indeed seem peculiar to list one of the world's major pest species along with other threatened lagomorphs (see Cooke 2008, this book). However, pest status for the European rabbit largely obtains when it is an invasive alien species, such as in Australia. In many areas of its native range, in particular the Iberian Peninsula, the European rabbit is in trouble (see Ferrer and Negro 2004; Moreno et al. 2004). In Portugal, population decline in the last 10 years has been estimated to be around 30%, and it is listed on the National Red List as near threatened (NT; Ferreira et al. 2004). The decline of the European rabbit has been attributed to disease and habitat loss. It is time for a careful geographic inspection of the population status of this species, and perhaps to initiate a Red List Assessment at the subspecies level (like *O. c. algirus* in southwest Iberia) to highlight those areas where this form is in greatest need of conservation attention.

Amami Rabbit (*Pentalagus furnessi*) - EN. The Endangered Amami rabbit occupies only two small islands with an area of occupancy of 335 km^2 in the

Ryukyu Archipelago in southern Japan. The only black rabbit, its habitat consists of broadleaf evergreen forests and cutover areas where perennial grasses dominate. The population size was estimated in 2003 at 2,000–4,800 in four habitat fragments on Amami Island, plus a small population on Tokuno Island. It is threatened by invasive mongooses, feral cats and dogs, as well as deforestation and development projects (Sugimura et al. 2000; Sugimura et al. 2003; Sugimura and Yamada 2004; Yamada 2008, this book).

Volcano rabbit (*Romerolagus diazi*) - EN. The Volcano rabbit is Endangered. It lives between 2,800–4,250 m in elevation in pine forests with a dense undergrowth of zacaton bunch grass in the Transverse Neovolcanic Belt surrounding Mexico City. Its distribution is restricted to four discontinuous areas of core habitat that together cover an area of around 280 km^2, and several of these are in turn highly fragmented. Habitat destruction (forest fires, logging, collection of zacaton for thatch) and hunting threaten the survival of the Volcano rabbit, and genetic inbreeding depression remains a concern (Romero and Velazquez 1994; Portales et al. 1996; Velazquez et al. 1996).

Swamp rabbit (*Sylvilagus aquaticus*) - LR(lc). While not currently Red-listed, Bond et al. (2004) indicate that the Swamp rabbit is becoming a matter of concern for a number of wildlife agencies in the United States. Their populations are apparently in decline, yet the species remains so poorly known that management decisions are difficult. Status surveys and monitoring of the Swamp rabbit are necessary.

Brush Rabbit (*Sylvilagus bachmani*) - LR(lc). While this species is designated as Least Concern, the subspecies *S. b. riparius* is US ESA Endangered and a candidate to be Red-listed as Critically Endangered. This form is found along streams of the lower San Joaquin River system in California. Here it occupies approximately 225 ha in two separate areas, one of which is highly fragmented. One population has declined to about 25% of its former size based on population censuses over the past 11 years. Some of its habitat is scheduled for urban development within the next decade. A third population has recently been established on historical habitat, but it is too soon to determine if the re-established population will be viable (Kelly et al. 2004; Williams et al. 2008, this book).

Mexican Cottontail (*Sylvilagus cunicularius*) - LR(nt). The Mexican cottontail, found along the Pacific coastal lowlands of Mexico, has been listed as Near Threatened, but further surveys are needed to determine the current status of the species. In some places competition with livestock, habitat fragmentation, fire, and volcanic activity represent threats to populations of the Mexican cottontail.

Dice's Cottontail (*Sylvilagus dicei*) - EN. Dice's cottontail is currently Red-listed as Endangered, but in reality there is insufficient information to conduct a thorough assessment of this species; its listing status should probably be changed to Data Deficient. This species is in need of further study.

Tres Marias Cottontail (*Sylvilagus graysoni*) - EN. Occupying four small islands off the coast of Mexico (the largest, Maria Madre is 145 km^2), the Tres Marias Cottontail is Red List Endangered, and considered critically endangered by the Mexican government. Nearly all the natural vegetation has been destroyed on two islands (Maria Madre and Maria Cleofas), and introduced *Rattus rattus*, white-tailed deer, and goats on Maria Magdalena (now considered an ecological reserve) compromises the health of the cottontail population there (Chapman and Ceballos 1990). This species is in need of monitoring and conservation action.

Omilteme Rabbit (*Sylvilagus insonus*) - CR. The Omilteme rabbit is the rarest and least understood of all lagomorphs. It is known definitively from only three specimens, and it has not been recorded in the wild since the early 1900s. Its area of occupancy is less than 500 km^2 in a semi-isolated mountain range in coastal western Mexico. Recently frequent intensive surveys failed to locate any Omilteme rabbits, leading to reports that this rabbit is extinct (MacPhee and Fleming 1999). In 1998, however, two rabbit specimens were collected and eaten by hunters, and the partial skin of one of these was recovered by Fernando Cervantes. This specimen was identified as an Omilteme rabbit, giving proof that the species is extant (Cervantes et al. 2004). While the Omilteme rabbit is listed also as critically endangered by the Mexican government and occurs in an ecological reserve, it remains at risk from hunting and habitat destruction. Clearly, we need more information concerning this species.

San Jose Brush Rabbit (*Sylvilagus mansuetus*) - LR(nt). The San Jose Brush rabbit is endemic to the island of San Jose (194 km^2) in the Gulf of California, Mexico (an ecological reserve). Red-listed as Near Threatened, the Mexican government considers the species as critically endangered. This animal is poorly known, but is apparently at risk due to introduced predators (cats and rats), as well as habitat loss (Chapman and Ceballos 1990).

Appalachian Cottontail (*Sylvilagus obscurus*) - LR(lc). The Appalachian cottontail is found in discontinuous upland shrublands and second growth (also recorded from old growth forest) from central Pennsylvania to northern Alabama (USA). It does not appear on the IUCN Red List, but is being proposed as a Vulnerable classification. This species is poorly known, but all available evidence indicates population declines, increasing fragmentation of occupied habitat, and reduced extent of occurrence and area of occupancy. The Pennsylvania Game Commission considers the Appalachian cottontail "at risk of becoming endangered" – and admits to a general lack of information. This species requires a thorough review.

Marsh Rabbit (*Sylvilagus palustris*) - LR(lc). This common game species is not threatened, but the Lower Keys Marsh rabbit subspecies, *S. p. hefneri*, is considered Endangered in the IUCN Red List and the US ESA. It is a candidate for Red-listing as Critically Endangered. This form occupies scattered isolated islands in the lower Florida Keys. Over the past 25 years, more than 50% of Lower Keys Marsh rabbit habitat has been lost to human development, and the form has been extirpated from several major islands it once occupied. The total population size is 300–400 individuals, with no key containing more than 250. Area of occupancy is less than 10 km^2, highly fragmented, and declining. Threats include habitat loss, habitat degradation (alien invasive species), fragmentation, and mortality from domestic and feral cats and dogs (Forys and

Humphrey 1999a, 1999b; Faulhaber 2003). A recovery plan has been developed and is being implemented by the US Fish and Wildlife Service (1990, 1999).

Robust Cottontail (*Sylvilagus robustus*) - NE. This species is found only on a few isolated mountains in western Texas, eastern New Mexico (USA) and northern Mexico. Long considered a subspecies of *Sylvilagus floridanus*, it has not yet been officially Red-listed; it is under consideration as Endangered. The species is isolated and its population is declining. Further monitoring of this species is needed (Ruedas 1998).

New England Cottontail (*Sylvilagus transitionalis*) - VU. The New England cottontail distribution consists of rare, never abundant, disjunct populations west of the Hudson River (New York) to southern Maine (USA). It occupies early successional forests and native shrublands, and these habitats are now being lost at a rapid rate or are becoming highly fragmented due to maturation of woodland habitat and development (Litvaitis and Villafuerte 1996). The species is also at risk of indiscriminate hunting (due to confusion with the introduced *S. floridanus*) or from competition with *S. floridanus*. It is suspected that its population size has declined by more than 50% during the past decade; the species may consist of fewer than 1,000 mature individuals. Many populations have already been extirpated (Litvaitis et al. 2003, 2008, this book). Thus, while the New England cottontail currently is Red-listed as Vulnerable, a listing of Endangered is being proposed.

Venezuelan Lowland Rabbit (*Sylvilagus varynaensis*) - NE. This newly described rabbit has not been previously assessed, and its status is poorly known. While it may currently be deserving of a Data Deficient classification, there are indications that it is a threatened form (Durant and Guevara 2000). A complete status survey needs to be conducted on the Venezuelan Lowland rabbit.

Ochotonidae (see also Smith et al. 1990; Smith 2008, this book)

Silver Pika (*Ochotona argentata*) - CR. The Silver pika occupies a very restricted area (2×1.5 km) in the isolated Helan Shan mountains in central China. Apparently its extent of occurrence and area of occupancy have decreased dramatically in recent years. This species is treated as Critically Endangered, but further observations on its status are needed, as well as recommendations for it conservation (Formozov 1997; Formozov et al. 2004).

Gansu Pika (*Ochotona cansus*) - LR(lc). The Gansu pika is common, mostly inhabiting the shrub belt at about 3,000–4,000 m, on the Tibetan Plateau. However, two extremely isolated subspecies exist, and these may be of conservation concern. *O. p. sorella* and *O. c. morosa* occur far to the east of the main species distribution. Of these forms, *sorella* has been Red-listed as Endangered, and *morosa* as Data Deficient. In reality, both should be Data Deficient, as I know of no surveys on these forms for several decades. Given their extreme isolation and the likely degradation of habitat throughout their area of occupancy, it is essential that survey and monitoring studies be undertaken on these two subspecies.

Forrest's Pika (*Ochotona forresti*) - LR(lc); Gaoligong Pika (*Ochotona gaoligongensis*) - DD; Tsing-Ling Pika (*Ochotona huangensis*) - EN (as *O. thibetana huangensis*); Muli Pika (*O. muliensis*) - DD; Black Pika (*Ochotona nigritia*) - NE; Thomas's Pika (*Ochotona thomasi*) - LR(nt). The true conservation status of these six pika species, all endemic to central China, is unknown. I know of no population or survey work ever conducted on any of these species, and most of these forms have not been reported by scientists for decades. They are listed here either because they are currently afforded a Red List category, or because they are candidates for a threatened status. The Black Pika was recently described and remains unstudied; it likely is a melanistic variant of *O. forresti*.

Hoffmann's Pika (*Ochotona hoffmanni*) - VU. This recently named species is Red-listed as Vulnerable. It occupies a restricted extent of occurrence in Mongolia (Bayan-Ulan Mountains) and Russia (Chita Province, Erman's Ridge; area 600 km^2; Formozov 1997; Formozov and Baklushinskaya 1999). To establish conservation action goals for this species, it needs to be surveyed and monitored further.

Northern Pika (*Ochotona hyperborea*) - LR(lc). The Northern pika has the largest geographic range of any pika extending from the Ural Mountains to the Pacific Ocean, and it is common in its preferred habitat throughout this area. However, the subspecies *O. h. yesoensis*, isolated on Hokkaido Island, Japan, is considered Rare in the Red List of Japanese Mammals. This form has recently been the focus of local conservation activity as development projects have been scheduled that would have seriously impacted critical pika habitat on Hokkaido.

Ili Pika (*Ochotona iliensis*) - VU. The Ili pika is currently Red-listed as Vulnerable, but we have proposed that it be re-classified as Endangered (Li and Smith 2005). There have been only 27 confirmed identifications of Ili pikas, plus about ten confirmed sightings by local pastoralists. This species occupies cliff-face habitat along two spurs of the Tien Shan Mountains in northwest China. Recent comprehensive surveys to known pika localities failed to find extant populations at approximately half the sites where they had been recorded 10–20 years previously; populations at the type and paratype localities had gone extinct. A combination of factors, exacerbated by the effects of global warming, have likely contributed to these losses (Smith et al. 2004; Li and Smith 2005). Continued monitoring of this species is necessary.

Koslov's Pika (*Ochotona koslovi*) - EN. The Endangered Koslov's pika occupies the isolated high mountains near the junction of Qinghai Province, Xinjiang-Uygur Autonomous Region, and the Xizang Autonomous Region in central China. Following its discovery, it was not seen again for about a hundred years (Zheng 1986). Recently, however, Li Weidong conducted surveys in the area and found that Kozlov's pika is locally abundant within its restricted geographic range. There is some evidence that its species range may extend farther to the west than previously thought (Formozov, personal communication; Li, personal communication). More survey work on this species needs to be conducted in this remote area, and conservation plans should be drafted and put into force.

Pallas's Pika (*Ochotona pallasi*) - LR(lc). As with the Alpine pika, within the otherwise common Pallas's pika are two highly isolated subspecies that are of conservation concern: the Critically Endangered *O. p. hamica* of Xinjiang-Uygur Autonomous Region, and the Endangered *O. p. sunidica* of Nei Mongol, China. I know of no recent surveys or population studies on either of these forms, thus they should properly be listed as Data Deficient.

American Pika (*Ochotona princeps*) - LR(lc). The American pika, found throughout mountains of the western United States and Canada, as a species is not of conservation concern; however, the species is divided into 36 subspecies and many of these and other populations are highly isolated and vulnerable to extinction. Hafner (1993, 1994) was the first to sample from all subspecies, and he noted that several historical populations were extirpated, leading to a Vulnerable classification for seven subspecies: *goldmani, lasalensis, nevadensis, nigrescens, obscura, sheltoni,* and *tutelata*. More recently several historical locations have been surveyed in the Great Basin of Nevada by Beever et al. (2003). They found that pikas had disappeared from 28% of 25 formerly known localities examined; subsequent surveys have demonstrated additional losses (Beever, personal communication). In addition, Nevada populations of two additional subspecies that extend into Oregon or California (*schisticeps, taylori*) may have been extirpated (Beever, personal communication). These losses have been attributed to global warming (in conjunction with the sensitivity of the physiology and population dynamics of American pikas to warm temperatures; Beever et al. 2003; Smith et al. 2004). It appears that low-elevation pikas are particularly susceptible to extirpation, and that pika populations may be a harbinger of the negative effects of global warming.

Steppe Pika (*Ochotona pusilla*) - VU. The Steppe pika has declined dramatically in historical time across Europe toward Asia. At the end of the Ice Age it spanned Europe, but it had retreated to the Ukraine by the 10th century, and to between the Don and Volga rivers by the 18th century. By the 19th century they lived only east of the Volga River. Nikolai Formozov (personal communication) has determined that several small populations remain in Europe in Russia's Saratov region. This retrenchment of the Steppe pika is due in part to climate change and in part to anthropogenic factors such as overgrazing, increasing intensity of agriculture and steppe fire. The Steppe pika is Red-listed as Vulnerable, however extant populations, primarily in Asia, may be healthy enough to downlist this species (Formozov, personal communication); a thorough Red List Assessment is necessary.

Moupin Pika (*Ochotona thibetana*) - LR(lc). While common throughout the majority of its range, an isolated subspecies of the Moupin pika, *O. t. sikimaria*, is Critically Endangered. The habitat of this form in Sikkim, India, has suffered serious degradation due to human settlement, agriculture, firewood collection and development projects (Smith et al. 1990).

Sardinian Pika (*Prolagus sardus*) - EX. The Sardinian pika once occupied Corsica, Sardinia and adjacent small islands in historical time. Fossil evidence indicates that it was present on Corsica and Sardinia within the last 2,000 years, and there is one report that may be referable to the Sardinian pika made in 1774. It is Extinct, presumably due to the combination of habitat loss, predation, and competition with alien invasive species—all resulting from the occupation of these islands by humans (Nowak 1991).

Conservation Action

IUCN-The World Conservation Union and its Species Survival Commission (SSC) Lagomorph Specialist Group (LSG) is entrusted with the conservation of the world's lagomorphs. The LSG, however, is comprised of volunteers and without a core budget. Its activities largely are to coordinate and support the conservation activities of its volunteer members and other biologists working to enhance the conservation and management of lagomorphs world wide. Many of these efforts are performed tirelessly and altruistically by individuals or small teams; in other instances local working groups or non-governmental organizations have been formed to carry out a mandate of conservation action and education. All of these efforts are laudatory and have provided much of the information for this review. I would like to highlight a few of the larger initiatives that have been taken by LSG members to further the cause of lagomorph conservation.

AMCELA – the Mexican Association for the Conservation and Study of Lagomorphs was founded in 1990 to address the pressing needs of lagomorph conservation in a country that is home to 15 species of leporid, many of which are threatened (see above; Portales et al. 2004). Many individuals have participated in this ground-breaking endeavor, but the early leaders were particularly visionary: Fernando Cervantes, Francisco Romero, Patricia Reyes and Alejandro Velazquez. In 1996, AMCELA hosted a CBSG/LSG combined Conservation Management and Habitat Assessment (CAMP) and Population and Habitat Viability Assessment (PHVA; on the Volcano rabbit) Workshop that heightened knowledge and awareness of threatened lagomorphs in Mexico (Portales et al. 1996). AMCELA engages in community-based conservation and extensive environmental education. This organization also serves to encourage young biologists, and its members generate significant data and information on the lagomorphs of Mexico. It is a model for local and country-wide conservation activity.

On the other side of the world, Victoria Ahlmann packed her bags and moved from Europe to South Africa to rescue the Riverine rabbit. She has forged a coalition of local government and non-government organizations, researchers, and landowners, into the Riverine Rabbit Working Group (Ahlmann and Collins 2004). As none of the Riverine rabbit's habitat occurs on public land, it is necessary to engage in significant public education and to form local conservancies to ensure that it does not go extinct. This activity represents another strong model for local species-based conservation.

The national parks on Hokkaido Island are not immune from major development projects, and many of these projects that have been planned would have destroyed key habitat of the Northern pika. Toshimi Ichikawa formed the Pika Fan Club, which has become one of the largest green groups in Japan, to bring attention to this problem, educate the public of the value of pikas and other native wildlife on the island, and to stop the planned expensive and unnecessary development projects that were on the drawing boards. The Pika Fan Club has succeeded in all these areas (Ichikawa 1999).

In summary, lagomorph diversity in the world is threatened, and it is necessary to take action to ensure its continuity in the world's ecosystems. There is much we do not know, and what little we do know about many of the species highlighted above is frightening. It will take resolve and hard work, and many more players than are currently active studying threatened lagomorphs, to complete this agenda.

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Managing the European Rabbit: Converging Interests Between Australian Research for Rabbit Control and European Research for their Conservation

Brian Cooke

Introduction

In Europe, European rabbits (*Oryctolagus cuniculus*) are a natural part of the fauna, or recognized traditionally as such, and their conservation is a primary management objective (see Letty et al. 2008, this book). Rabbits are a key grazing species in the Mediterranean shrub lands of Spain, Portugal and southern France and they are the primary prey of endangered predators such as the Imperial Eagle *Aquila adalberti*, and the Iberian Lynx, *Lynx pardinus*. Rabbits are also important as wild game and support major hunting industries, in France, Portugal and Spain. By contrast, in Australia and New Zealand, rabbits are recognized pests and are vigorously suppressed. Despite these opposing aims, however, international collaboration has grown over the last 25 years and has greatly increased our knowledge of the management of European rabbits. This collaboration was stimulated by Ken Myers and Charles MacInness who organized the 1st World Lagomorph Conference held in Guelph, Canada, in 1979. Contacts made between scientists at that time led to very useful advances in rabbit research and the 2nd World Lagomorph Conference in Vairao, Portugal, 26–30 July, 2004, provided an opportunity to review some of the progress made as well as looking towards future progress.

History and Development of Rabbit Control Methods in Australia

European rabbits were introduced into Australia from Britain in 1859 and within 70 years had become widespread across the southern two-thirds of the continent. They caused enormous problems for agriculture by eating crops, damaging pastures used for cattle and sheep production and making the land more vulnerable to soil erosion (Ratcliffe 1948).

A startling array of control methods was soon developed including poisoning, by using arsenic or strychnine applied on baits, gassing of burrows using

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carbon monoxide or cyanide and the digging out of rabbits and destruction of their burrows. To halt the advance of rabbits, wire netting fences were erected and were also used to keep individual properties rabbit-free.

Nevertheless, the introduction of the myxoma virus into Australia's European rabbit population in 1950 (Fenner and Ratcliffe 1965) was a major breakthrough for rabbit control in Australia. It caused 99% mortality among infected rabbits and at first was highly effective in keeping rabbit populations down. This experience also opened up the way for rabbit control to be considered using detailed scientific methods.

Myxomatosis—a Critical Turning Point in Australia's Battle with the European Rabbit

Ken Myers was a member of Francis Ratcliffe's team involved in initial field trials of the myxoma virus and after its spread into the Australia's European rabbit population he collaborated directly with Frank Fenner in epidemiological studies. However, it was recognized very quickly that the myxoma virus was attenuating into a wide range of field strains, generally with lower virulence than the virus that was initially released, and that rabbits were also developing genetic resistance to the virus. As a result, Myers and his co-workers became increasingly involved in studies to see what could be done to curb the recovery of European rabbits across Australia as the initial impact of myxomatosis waned (Poole 1960; Rowley 1957). Myers also carried out studies on penned European rabbits as well as major field studies on the biology of European rabbits, noting the variations in productivity of rabbits in different climatic regions (arid, alpine, sub-tropical and Mediterranean-like areas) within Australia (Myers 1971).

Parallel studies by Mykytowycz (1958, 1959, 1960, 1961) mainly on rabbit behaviour and social organization also added to the growing background knowledge of rabbits and these studies were continued by other scientists (e.g. Dunsmore 1966; Daly 1981; Wood 1980; Parer and Fullager 1986; Williams et al. 1995) with increasingly sophisticated experimental designs as they sought answers to questions about the impact of myxoma virus despite attenuation, the role of parasites in population control, the impact of predation, or simply tested new or better combinations of rabbit control methods.

Interestingly, the spread of the myxoma virus through Europe after its release in France in 1952 was followed up by relatively few epidemiological studies despite its impact on rabbit populations. Nevertheless, in the UK studies by Ross and Sanders (1984, 1987) showed that rabbits gradually accumulated genetic resistance to the attenuating myxoma virus albeit more slowly than was the case in Australia.

In Australia today, poisoning, fumigation and warren destruction continue to be used although continuing research and development has helped in the selection of chemicals such as the poison monosodium fluoroacetate (1080) to minimize effects on other non-target species. Given that 1080 occurs naturally in some Australian plants (e.g. *Gastrolobium* spp.) Australian wildlife species that evolved alongside such plants have considerable tolerance to this toxin (Oliver et al. 1979). Knowledge of the territorial behaviour of rabbits also assisted in determining how to lay poison baits effectively and in recent years there have been great improvements to methods for destroying rabbit warrens: powerful bulldozers with winged tines are now used to rip up rabbit warrens with great efficiency although such methods continue to be reviewed (Edwards et al. 2002) Combinations of techniques, such as poisoning followed by warren ripping and fumigation have also proven highly effective (Cooke 1981; Williams et al. 1995).

Despite this increasing knowledge and increased benefits from programs organized in each Australian state to apply the best technology for rabbit control, the application of mechanical and chemical rabbit control methods has always been limited by the costs, especially in those areas where agricultural production is low and the economic benefits from rabbit control are small. Consequently, it was not surprising that the next innovative step towards rabbit control in Australia arose in the field of self-disseminating biological control agents when Sobey and Connelly (1969) introduced European rabbit fleas, *Spilopsyllus cuniculi*, in late 1968 as new vectors for myxomatosis.

Introduction of Rabbit Fleas

The introduction of the European rabbit flea into Australia followed fascinating work in the UK by Mead-Briggs (1964), Mead-Briggs and Vaughan (1969) and Rothschild and Ford (1964, 1973) who had unravelled the breeding biology of the European rabbit flea, showing that fleas were stimulated by hormones in female rabbits during late pregnancy, preparing them for mating and egg-laying on new-born rabbit kittens. This knowledge made it clear that *S. cuniculi* was a specific parasite of the European rabbit and it also enabled colonies to be maintained in the laboratory. Along with evidence that the fleas were the main vector of myxomatosis in Europe there was mounting evidence that their introduction into Australia could improve the transmission of myxomatosis, particularly in dry areas where mosquitoes were inadequate vectors. The fleas were introduced experimentally and they facilitated more rapid disease transmission in some areas, especially where mosquitoes were not abundant. Unfortunately, the European rabbit fleas proved unable to colonise arid, inland Australia (Cooke 1984, 1990a). Consequently, arid-adapted fleas were sought in southern Spain where rabbits were known to be hosts to at least four species of fleas (Beaucournu 1980). Collaborative work began with J.-C. Beaucournu, Université de Rennes in France and R. Soriguer, CSIC Estación Biologica de Doñana in Spain but eventually grew to include a number of other European contributors.

From three different species of rabbit fleas studied in eastern Spain, *Xenopsylla cunicularis,* was finally chosen (Cooke 1990b) and work began toward importing them into Australia through quarantine. This work involved assessing the risk of using these fleas in Australia, by testing them on 25 species of native Australian mammals as well as understanding the distribution and ecology of both rabbit fleas and those from native Australian mammals. Resultant scientific papers, by European and Australian scientists, include descriptions of little known fleas from hairy-nosed wombats (Beaucournu et al. 1990) and two new species of mites that infest fleas (Fain et al. 1990).

Permission was granted to release the imported rabbit fleas from quarantine in 1991, almost a decade after the work first began. Methods for mass-rearing the fleas had been developed during the initial years of research and in a number of laboratories across Australia over 1 million rabbit fleas were eventually bred for release and distributed widely throughout the arid zone in particular. Today the fleas probably assist the spread of myxomatosis across a large part of inland Australia although their efficacy was impossible to establish given that soon after their establishment rabbit haemorrhagic disease also spread with major impact.

While these studies were under way in Australia, there was increasing interest in rabbit fleas in Spain. Beaucournu (1980) had carried out a revision of the fleas found on rabbits and had gathered evidence that the fleas occurred in distinct sub-species, possibly reflecting their isolation on sub-populations of rabbits during previous glacial periods. J. Lucientes in the Veterinary School in Zaragosa and his students began studies on the fleas (Osacar et al. 2001) with an initial aim of considering ways in which fleas could be reduced to slow the spread of myxomatosis as had been done experimentally in the UK (Trout et al. 1993). Subsequently, the idea of using fleas to transmit a recombinant myxoma virus (MV) expressing the rabbit haemorrhagic disease virus (RHDV) capsid protein capable of immunising rabbits against both myxomatosis and rabbit haemorrhagic disease has also been explored (J. Lucientes, pers. comm.; Barcena et al. 2000).

Rabbit Haemorrhagic Disease

During the studies on rabbit fleas in Spain, the first outbreak of rabbit haemorrhagic disease (RHD) was recorded in 1988 among European rabbits in Almería, in south-eastern Spain (Rogers et al. 1994; Cooke and Fenner 2002). The causative rabbit haemorrhagic disease virus (RHDV) had been introduced into Spain in imported rabbit meat and had begun to affect European rabbits as well as domestic ones. L. Leon-Vizcaino (unpublished) initiated the first epidemiological studies on the rabbit haemorrhagic disease virus (RHDV) in Murcia, south-east Spain and other research on RHD in Spain and other parts of Europe was followed closely in Australia and New Zealand where the potential of the disease as a rabbit control agent was recognized at an early stage (Cooke 2002).

RHDV was imported into high security quarantine facilities in Australia in 1991 and experimental work, including assessing native fauna for susceptibility to the virus was carried out over 4 years (Lenghaus et al. 1994). Once satisfied that the virus was most unlikely to affect species other than the European rabbit, approval was given for field trials on Wardang Island, South Australia. This work was done to assess the ability of the virus to spread under the relatively severe climatic conditions of southern Australia.

Wardang Island had been used for testing the myxoma virus some 50 years earlier, and that work had been completed without incident. Furthermore, RHDV, at the time, was considered to spread mostly by direct rabbit to rabbit contact. Even so, considerable care was taken to minimize the risk that insects might carry the virus off the island. Despite all precautions however, the virus escaped from quarantine pens on the island (Fenner and Fantini 1999) and subsequently became established on the Australian mainland and spread very rapidly (Kovaliski 1998).

The escape of RHDV precipitated extensive epidemiological studies in the field and rabbit population studies were carried out in collaboration with L. Capucci and A. Lavazza, OIE World Reference Laboratory for RHDV, Brescia, Italy, who supported the work with ELISA techniques (Capucci et al. 1995), which enabled a deeper understanding of the dynamics of the disease in the rabbit population (Robinson et al. 2002a, 2002b).

The contacts set up between Australia, Italy and Spain have led to a major exchange of ideas such as the development of methodologies for surveying the spread and epidemiology of RHD. For example, Villafuerte et al. (1995) showed that large scale questionnaire surveys of hunters could provide information on the impact of RHD in different climatic regions of Spain and subsequent questionnaire surveys in Australia involving regional rabbit control experts and farmers have also given useful insights into regional variations in the epidemiology of RHD. In the state of New South Wales, RHD seems to have had far greater effect in the arid south-west of the state where outbreaks mainly occur in winter and it has been less effective in the north-east where outbreaks occur mainly in summer. The underlying factor driving these epidemiological patterns appears to be related to seasonal rainfall patterns in particular.

Significant progress has also been made in developing a theoretical understanding of the epidemiology of RHD. For example, Calvete and Estrada (2000) developed epidemiological models for RHD and proposed that its impact in reducing rabbit populations was inversely related to the density of rabbit populations, arguing that in dense populations where the virus spreads quickly, rabbits were relatively young when infected and more likely to recover given that RHD is more acute in older rabbits. This idea has subsequently been tested experimentally by Greg Mutze and . . . McPhee (unpublished) in Australia with a broad conclusion that rabbit density does indeed influence the rate of spread of RHD. However, much more work needs to be done to establish whether these density effects are sufficient to explain the wide site to site variability in the epidemiology of RHD in either Europe or Australia. Thus, the development of a sound epidemiological model for RHD still remains of great importance both for the continuing control of rabbits in Australia and for reducing its effects on rabbit populations in Europe.

Genetically Modified Myxoma Viruses

In Europe, in recent years, a recombinant myxoma virus has been developed into which genetic material encoding the coat protein of RHDV has been inserted. The myxoma virus was selected for its low virulence so that it effectively immunizes rabbits against both myxomatosis and RHD (Barcena et al. 2000). In parallel work in Australia, recombinant myxoma viruses containing genetic material encoding zona pellucida proteins of the rabbit's ovum have been sought to sterilize rabbits (Robinson et al. 1997) on the logical and humanitarian grounds that it would be better to prevent rabbits being born that to continue using lethal viruses to eliminate sub-adult rabbits.

Unfortunately, if either virus were spread or deliberately introduced to inappropriate parts of the world, results would be unpredictable and could possibly reverse gains in rabbit control or undo conservation efforts. This too has been an issue that requires interchange of ideas between Australasia and Europe in trying to regulate the development and use of recombinant viruses (Angulo 2001; Angulo and Cooke 2002). These publications helped stimulate an international symposium on the use and control of GMOs in managing rabbits and other mammals. The symposium, held in New Zealand, was organised by Robert Henzell (Animal and Plant Control Commission, South Australia) and Elaine Murphy (Department of Conservation, New Zealand), with major papers contributed by Juan Barcena (CISA-INIA, Spain) and Roger Trout (Forest Research, UK). A summary of that symposium is available (see Cooke et al. 2004).

Future Collaborative Research

With modern transport and communications as well as limited science budgets, it seems inevitable that collaborative research must continue to grow between those countries where the management of European rabbits is important. The motives for research will be different but the fundamental understanding of factors limiting rabbit populations is of critical importance in each case.

Scientific interest in myxomatosis and RHD has grown noticeably in recent years, largely because the European rabbit population has been seriously reduced by the combined impact of both diseases. Furthermore, traditional methods of rabbit management for maintaining adequate populations were frequently based on small hunting reserves and relied on reduction of predation and maintenance of suitable habitat for rabbits. However, with more competing pressures on land use such methods are now apparently inadequate for maintaining rabbit populations, especially on the scale needed to support endangered species such as the Iberian lynx and the Imperial eagle.

Current research is providing a deeper understanding of the way in which viruses work. For example, Ruvoën-Clouet et al. (2000) found that entry of RHDV into rabbit cells involves ABH antigens, a mechanism in common with other caliciviruses including those affecting humans (Huang et al. 2003) and similarly, Lalani et al. (1999) reported that chemokine receptors on cells are probably the entry point for myxoma virus, although it has since been shown that this binding also has a role in inhibiting the immune response to infection (Seet et al. 2001). Detecting mutations or changes in gene frequency of ABH and chemokine receptors in rabbits may be a means of measuring evolution of disease resistance in European rabbits. S. Marchandeau and J. Le Pendu have begun population studies on the frequencies of the genes producing ABH antigens in European rabbits to see whether there are differences in rabbit populations related to the frequency of their of exposure to RHDV.

There have also been other new developments. For example, the rabbit trypanosome, *Trypanosoma nabiasi*, has recently been discovered in Australian European rabbits (Hamilton et al. 2005), raising questions about its arrival in Australia, especially since it is believed to be transmitted only by the European rabbit flea *S. cuniculi*, introduced into Australia as recently as 1969. Modern molecular techniques (e.g. PCR) enable its detection both in the blood of infected rabbits and in the fleas that are intermediate hosts. It would be interesting to record the distribution of these trypanosomes in Australia given that the European rabbit flea is limited by climatic constraints to only part of the rabbit's range. This new organism also raises some prospects for future rabbit control. *T. nabiasi* is considered to be non-pathogenic, but, as some other trypanosomes can be maintained in culture medium and some have been modified genetically, it is worth asking whether *T. nabiasi* might be considered in the long run as a potential GMO for managing rabbits.

Conclusions

This brief review has indicated a steadily increasing collaboration in research into rabbits and sharing of information and technology between European and Australian researchers whose interests lie, directly or indirectly in the management of rabbit populations. This has occurred despite researchers in Australia having the very different objective of rabbit control in contrast to the increasing importance of conserving rabbits in Europe. The common element is the importance of having a broad science base to underlie all management decisions. The stage seems set for continued research and the sharing of information on the management of myxomatosis and RHD between Europe, Australia and New Zealand. This will include not only epidemiological studies and modelling of populations but also considerations of issues such as the management of genetically modified organisms as new ideas for rabbit management are explored.

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Improving Rabbit Restocking Success: A Review of Field Experiments in France

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Introduction

European Rabbit Status in South-Western Europe

Restocking is now a common practice in management of European rabbit *Oryctolagus cuniculus* in France, Portugal and Spain, following the decline of this species that occurred during the second half of the 20th century. This population decline was mainly due to the global change in the countryside caused by intensification of agriculture in temperate regions and conversely decline in Mediterranean regions, and to the appearance of new epizootics, myxomatosis in the 1950s (Ross and Tittensor 1986), and rabbit hemorrhagic disease in the 1980s (Marchandeau et al. 2000a). In addition, there has often been unsustainable hunting management of European rabbits. The extent of the population decline is shown by the French national bag records survey: 13.5 million rabbits in 1974/75, 6.4 million rabbits in 1983/84 and 3.2 million rabbits in 1998/99 (Arthur and Guénézan 1986; Marchandeau 2000). The European rabbit is not actually endangered, but many of its populations are still declining and cannot support sustained harvesting, which is problematic for hunting and for the conservation of rabbit-specialist predators such as the Iberian lynx (*Lynx pardinus*), the Imperial eagle (*Aquila adalberti*), or even the Bonelli's eagle (*Hieraaetus fasciatus*). Therefore, hunters and wildlife managers have promoted rabbit restocking so that in south-western Europe, around half a million rabbits are translocated each year from large natural populations or breeding centres. However, the success of rabbit restocking is known to be generally low (Arthur 1989; Mauvy et al. 1991; Calvete et al. 1997; Letty et al. 2002a).

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Translocations Problematic

It is not very surprising to get low restocking success in European rabbits as this is also found in many other animal species (Letty 1998). Translocation success requires achievement of several steps: the survival of the released individuals, their settlement in the release area, their breeding success and the viability of their offspring. The translocation process itself is also a severe test for individuals as they are removed from their population and environment of origin to repopulate an unfamiliar area or to reinforce another population. Therefore, it is likely that the translocation will induce short- or long-lasting biological effects for the transferred individuals, and that, in consequence, they may have unsuitable behaviours or low demographic parameters. The released individuals are affected by the stresses of capture or handling, and the novelty of the environment that inevitably occur in a translocation, but may also be affected by other problems such as social disturbance, low genetic diversity in the founder population, maladapted behaviour, or habitat unsuitability (Lyles and May 1987; Curio 1996). If the released individuals cannot cope with these problems, then the overall outcome of the restocking may be jeopardized. However, restocking success may also depend on some characteristics of the translocated individuals (body condition, age or social status), on their population and environment of origin that shaped their natural skills, or on the translocation protocol.

We carried out several field experiments in order to improve our knowledge of factors affecting rabbit restocking, and to get a better understanding of the prerequisites for its success. Here we present a review of our studies of rabbit restocking in France, and also of results recorded by other research teams on translocation of European rabbits, hares and other vertebrate species.

European rabbit Restocking

Materials and Methods

We conducted our field studies using methods that hunters commonly use to restock European rabbits, or at least, methods that they could easily use. However, we followed an experimental design to test hypotheses about factors that may determine rabbit restocking success or improve the release protocol (Armstrong et al. 1994a; Soderquist 1994; Marchandeau et al. 2000b). In general, we captured adult European rabbits by ferreting warrens in winter. Each rabbit was kept in a small individual wooden box and given a piece of beet or of apple as a source of wet food to limit dehydration. Before transportation by car, all rabbits were weighed, sexed, and vaccinated against rabbit haemorrhagic disease and myxomatosis. We also marked them with ear-tags covered with reflective paper suitable for long-distance

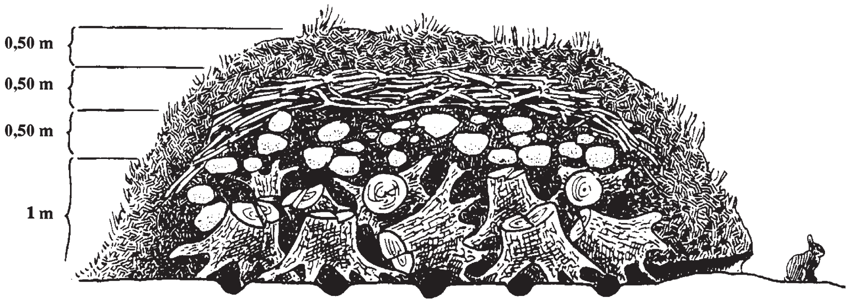

Fig. 1 Drawing of a release warren made of a big pile of stumps and of stones covered with loam and branches (designed by M.-A. Aubineau)

identification, particularly when using spotlight by night; each individual had a distinctive combination of colours. In some studies, we also radio-equipped some rabbits, using transmitters supplied with a loop antenna and a mortality sensor (Biotrack TW-5 or Televilt TXP-1, range: ca. 1.5 km; weight: ca. 30 g, 2–3% of the body mass). We analysed survival data following standard capture-mark-recapture (CMR) methods (Lebreton et al. 1992) using, for instance, the "known fate" procedure of the software MARK (White and Burnham 1999) with telemetry data. In particular, we used the likelihood ratio test (LRT) to assess hypotheses.

Rabbits were released on the day following capture, during the morning to avoid the nocturnal period of activity of the species. In each study area of 5–50 ha, several artificial release warrens were made of a big pile of stumps covered with loam and branches (Fig. 1). A group of 3–10 rabbits, males and females mixed according to the sex-ratio at capture, was released in each warren, irrespective of the warrens in which they were captured, so that previous social links were probably broken off as in a traditional restocking. Rabbits were then monitored during several weeks after release either by spotlighting at night when they forage or by radio-tracking to locate their diurnal resting places. In some studies, the release warrens were fenced with wire mesh during the first few days after release, so that rabbits were kept inside temporary pens for an acclimatisation period before discovering the new environment (soft release vs. hard release); some food was provided inside pens. However, some rabbits succeeded in escaping on their own from pens before fence removal. Details of the different studies are provided in Table 1.

Features of Traditional Rabbit Restocking

We first wanted to determine why rabbit restocking success is generally low. Therefore, during a traditional winter restocking conducted by hunters, we monitored rabbits after the release in order to know their fate and their behaviour, and the reason why they vanish (study W-a1, Table 1; Letty et al. 2002a).

Table 1 Rabbit restocking studies: study code, season, year, release area rural district, translocation protocol (hard release: no acclimatisation; soft release: after acclimatisation in pen; familiarity: individuals captured in a same warren; tranquillisation: injection before transport), age class, numbers of captured and radio-tracked rabbits, and body mass range (m: radio-tracked; m*: all released). Environments of origin and release areas are indicated (*small letters* refer to distinct areas). Park: temperate (northern France), or semi-temperate (*: Causses, Massif Central, France), clayey areas, grasslands, woodlands. Sand: temperate sandy areas (western France), grassland, dunes. Bocage: clayey temperate areas (western France), pastures, cereals fields, hedges. Mediterranean: scrubland, small fields (southern France). Rabbits were wild-caught, except 'S-d' rabbits that came from a breeding facility in semi-natural conditions

STUDY Date, Release area	Translocation protocol Experimental - release design	Individuals (- transport death) Radio-tracked ind. Body mass range	Environment	
			Origin area	Release area
W-a1: Winter 1996, Fay-de-Bretagne	Hard release, Familiarity within groups/ intermixing: 8 groups, balanced group design	51 (−2) adults 29; m *: 1,160–1,720 g	Park	Bocage[a]
W-a2: Winter 1997, Fay-de-Bretagne	Hard release, 4 groups	28 (−1) adults 27; m : 1,010–1,600 g		
W-h: Winter 1997, Héric	Hard/soft release (3 days), Tranquillisation/none: 16 groups, crossed balanced group design	109 (−5) adults none; m *: 1,000–1,740 g	Park[k]	Bocage[a]
W-g: Winter 1998, La-Chevallerais	Soft release (3 days), Familiarity within groups / intermixing: 8 groups, balanced group design	54 (−1) adults none; m *: 1,100–1,610 g	Park[k]	Bocage
W-control: Winter 1998, Cerizay	Control experiment: Short handling / storage in box (1 day) / storage in box and transport (1 day), Hard release in warrens of origin, 15 groups, group design	49 adults none; m *: 1,100–1,790 g	Bocage (no translocation: origin area = release area)	
S-d: Summer 1998, Dompierre-sur-Yon	Soft release (7 days), 5 groups	50 (sub-)adults 40; m : 800–1,270 g	Bocage, breeding enclosure	Bocage
S-e: Summer 1999, La-Ferrière	Soft release (9 days), 5 groups	55 (sub-)adults 35; m : 750–1,610 g	Park *	Bocage
S-f: Summer 2000, Dompierre-sur-Yon	Soft release (5 days), 4 groups	17 (sub-)adults 17; m : 1,100–1,550 g	Sand	Bocage

(*Continued*)

Table 1—Continued

STUDY Date, Release area	Translocation protocol Experimental - release design	Individuals (- transport death) Radio-tracked ind. Body mass range	Environment	
			Origin area	Release area
W-b: Winter 2001, Dompierre-sur-Yon	Soft release (5 days), Storage condition in box: alone / small group 6 groups, intermixing design, 2 batches	35 (−1) adults 2 (−1) juveniles 27; m : 1,210–1,810 g	Sand[l]	Bocage
W-c: Winter 2002, Dompierre-sur-Yon	Soft release (5 days), Storage condition in box: alone / small group 5 groups, intermixing design, 2 batches	17 (−1) adults 16; m : 1,340–1,860 g	Sand[l] Bocage	Bocage
S-c: Summer 2002, Dompierre-sur-Yon	Soft release (2 days, group escape), 1 familiar group	7 adults, 1 juvenile 7; m : 1,400–1,940 g	Bocage	Bocage[c]
W-i: Winter 2003, Aurons	Hard / soft release (9 days), 7 groups, group design	30 adults 30; m : 1,220–1,660 g	Blaigowrie[m], Scotland	Mediterranean
W-j: Winter 2003, Martigues	Soft release (9 days), 1 group	24 adults 15; m : 1,420–1,850 g	Blaigowrie[m], Scotland	Mediterranean

Among 49 individuals that were released, we recorded a mortality rate of 50% during the first 2 days after release (Fig. 2); there was no difference between sexes ($P_{LRT} > 0.4$). Moreover, it was obvious that mortality was not evenly distributed among the several release groups, which probably reveals micro-environmental differences among release warrens. Predation by mammalian carnivores was apparently the main cause of mortality, as 13 of the 18 dead rabbits recovered during the first month from release were found buried or eaten. However, mortality caused directly by stress could not be discarded in some cases as, for instance, two rabbits died during transportation, and three others died in the warrens after release. There were also two road kills. An adverse effect of radio-tagging on survival could be discarded, as mortality was equivalent between tagged and non-tagged rabbits. After the first 2 days, survival appeared to be quite normal. During the first week, most of 17 radio-tracked individuals displayed a quite intense movement activity (Fig. 3). However, three of them stayed very close to their release warrens. Moreover, there was no difference in distance from release warrens between males ($n = 7$)

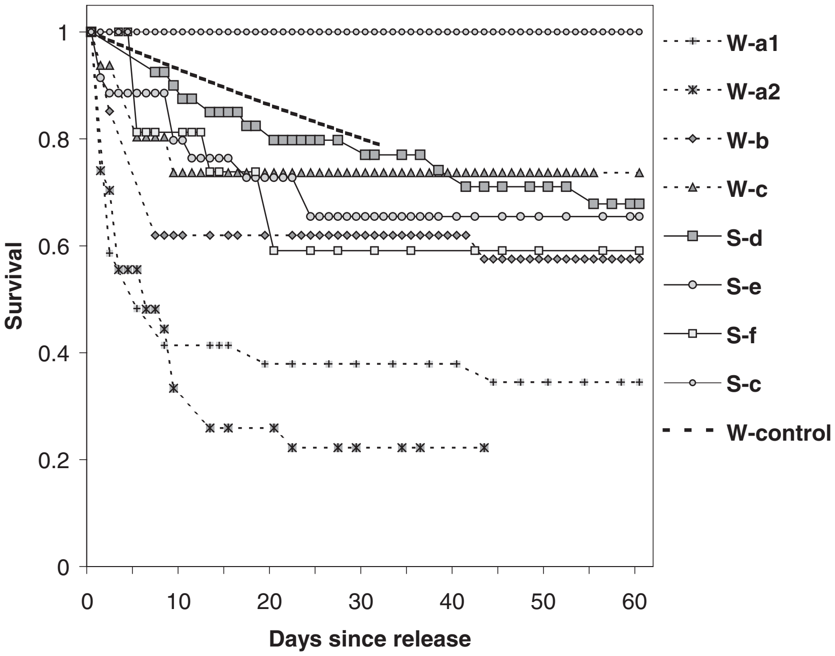

Fig. 2 Survival rate after release of translocated rabbits, at least 3 months old, recorded during several winter (**W-**) or summer (**S-**) restocking studies conducted in western France (*small letters* refer to distinct release areas, and *numbers* to distinct years). Survival curves are derived from known-fate data (radio-tracking), and mortality induced by radio-collar and signal losses were both considered as 'losses on capture', and not as real mortality. The control study survival curve is derived from resighting data modelling (W-control). Each study is described in Table 1

and females ($n = 10$) whatever the radio-tracking interval ($P_{t\text{-test}} > 0.1$). The largest dispersal movement was around 1 km away from the release warren, and, interestingly, the average distance between resting places and release warrens was greater 2 days after release than 3 days later on, suggesting a return of dispersers. In the long term, rabbits finally settled down at around 200 m away from their release warrens, on average.

The high mortality early after release clearly appeared as the first major problem for rabbit restocking success, whereas dispersal movements were revealed to be only moderate, suggesting that restocking viability can be promoted by releasing rabbits in neighbouring warrens. These results were in agreement with those from Spain of Calvete et al. (1997) and Calvete and Estrada (2004). Moreover, these authors have noted the absence of difference between sexes for both survival and dispersal after release, which also agrees with the results that we have recorded in other restocking studies (Letty et al. 1998, 2002b, 2005). Such a high early mortality reveals the great vulnerability of the rabbits once they have been translocated into their new range,

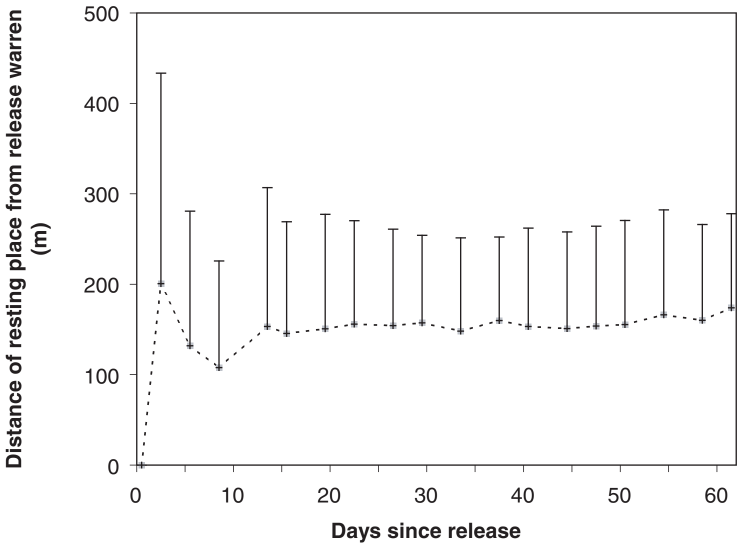

Fig. 3 Mean distance (m, ±SD) of diurnal resting places from their release warrens of translocated rabbits, at least 3 months old, located by radio-tracking during the days following the release (study W-a1, Table 1). Data sample size decreased over time from release (day 2: 16; day 5: 13; day 8: 11; day 19–61: 10); an out-layer, a female that dispersed 1 km away, is not included

particularly in respect with resident predators. Precisely, among the 88 radio-tracked rabbits that died from a "natural" cause during the first month after release in our studies (Table 1), predation was implicated in 69% of the cases. But, a spontaneous mortality seems to have occurred in 26% of the cases (intact carcass or death within a release warren), without taking into account the previous mortality during transportation (around 2%); there were also 5% of road kills. Therefore, we suspect that the early mortality after release could result above all from both the environment novelty and a probable physiological stress induced by capture and transportation, which could result in a significantly increased predation risk. Moreover, the initial increased movement behaviour of translocated individuals in the new and unfamiliar environment might increase mortality by putting them at risk of predation (Metzgar 1967; Ambrose 1972; Snyder et al. 1976; Sievert and Keith 1985; Ebenhard 1987). High mortality after translocation has also been recorded in several hare species (Schultz 1980; Lemnell and Lindlöf 1982; Sievert and Keith 1985; Marboutin et al. 1990; Angelici et al. 2000), and in many other vertebrates (O'Bryan and McCullough 1985; Massot et al. 1994; Mayot et al. 1998). Dispersal after release does not seem to be a major problem for rabbit restocking success. Indeed, among the 193 rabbits that we have located after release by radio-tracking in our studies (Table 1), 80% always had their resting place less than 300 m from their release warren, and only 2% dispersed beyond 1 km away, and up to 1,500 m away. Most of the large movements occurred early after release. This is another characteristic of translocations that has often been noted in birds and mammals (Davis 1983; Bright and Morris 1994; Castro et al. 1994; Soderquist 1994; Sjöåsen 1997), including hare species (Sievert and Keith 1985; Marboutin et al. 1991). In fact, these early movements might be motivated by homing behaviour, some individuals trying to go back to their home range, as has been recorded after translocation in both birds and mammals (Booth 1988; Clarke and Schedvin 1997; van Vuren et al. 1997; Ruth et al. 1998; Michallet and Toïgo 2000; Cowan 2001; Clark et al. 2002). Occasionally, large movements of rabbits also occurred a few months after release (unpubl. data). Finally, the distance from the release site at which the rabbits settled appears to be concordant with the natural dispersal range of the species (Cowan 1991).

Impact of Stress Due to Translocation Handling?

A first hypothesis to explain the high and sudden mortality after release is that capture, storage, and transportation induce a strong physiological stress that handicaps rabbits once released, in particular through an increased predation risk. Indeed stress is a well known problem in animal management (Hofer and East 1998; von Holst 1998). We carried out several studies to investigate this hypothesis and various ways to avoid these possible problems.

We first tested the effect on survival of tranquillising rabbits during handling and before transportation in order to limit the related stress (study W-h, Table 1; Letty et al. 2000). Tranquillisation consisted of two intra-muscular injections of a tranquilliser, respectively just after capture and just before transportation. There were 49 tranquillised rabbits and 55 control rabbits that were not tranquillised. The tranquilliser used, carazolol, a β-blocker, moderates the cardiac and respiratory rhythms under stressful conditions, and is prescribed in rabbit farming to decrease cardiac mortality and loss of body mass during transportation. There was no sleepiness in tranquillised rabbits at release. Finally, there was no effect of tranquillisation on survival after release ($P_{LRT} > 0.4$). However, as overall survival was atypically high in this study, around 80% over the first 12 days after release, a possible effect of tranquillisation might have been in fact overridden by other undetermined and uncontrolled factors.

However, a low level of stress due to handling could also explain the absence of effect of the tranquillisation treatment. To check this hypothesis, and to avoid a mix-up with the effect of environment novelty, which is inevitable during an actual translocation, we captured, handled, and transported rabbits before releasing them back into their own home range (study

W-control, Table 1; Letty et al. 2003a). Then, we could assess the effect of translocation handling alone, and we compared the fate of rabbits which were released either just after capture ($n = 12$), or after one-day-long restraint in box following transportation by car ($n = 19$) or after one-day-long restraint in box without transportation ($n = 18$). Survival after the rabbits were released back into their home range was very high, around 80% over 1 month (Fig. 2), and there was no difference among the three handling treatments ($P_{LRT} > 0.7$). Moreover, the mortality was not significantly increased just after release ($P_{LRT} > 0.1$). Thus, translocation handling did not seem stressful enough to induce mortality.

Another study designed to assess the effect of storage conditions during translocation on the short-term survival of rabbits after release confirmed that handling stress was probably not a tremendous problem (studies W-b and -c, Table 1; Letty et al. 2005). We compared survival after release of rabbits that have experienced one of two different restraint treatments in box between capture and release: either alone ($n = 29$) or in group of four or five individuals ($n = 25$). However, there was no clear difference in survival between the two treatments ($P_{LRT} > 0.1$), and the overall survival over the first 10 days after capture was of 61%. This suggests that storage conditions were not a determining factor for release success in European rabbits.

There is likely an acute stress in most animal translocations, as stress may be rapidly induced by handling (Hamilton and Weeks 1985; Waas et al. 1999). For instance, storage is a stressful event that may induce the death of some individuals (Guthrie et al. 1967; Mazurkiewicz 1968), and we recorded in rabbits a mortality of around 2% during one- or two-day-long storage in our studies (Table 1). However farmed rabbits are known to be little affected by transport, but much more affected by cage changing, which is probably related to rabbit territorial behaviour (Finzi and Verità 1980; Verità and Finzi 1980). Moreover in European rabbits, our data suggests that handling stress is apparently not strong and long-lasting enough to induce mortality when individuals come back home, as if stress suddenly vanished, whereas there is a high mortality after an actual translocation. There are some concordant data in snowshoe hare *Lepus americanus* (Sievert and Keith 1985). The apparent absence of effect of both tranquillisation and storage conditions backs up the idea that the handling stress is not insurmountable for translocated rabbits. However, an acute stress due to handling certainly exists in European rabbits as revealed during a quarantine monitoring in individual cages commonly used for rabbit breeding (Calvete et al. 2005). These authors report a mortality rate of 12% within the first 23 days, significant changes in physiological parameters, and an initial and transitory loss of body mass. In addition, all pregnant females aborted or lost litters. However, despite the reality of this stress induced by handling, our results suggest that the main problem for the survival of translocated rabbits may actually be the change of environment.

Problems Linked to the Change of Environment: Novelty and Quality of the Release Area

The first problem that individuals inevitably face after translocation is the novelty of environment, which may be another important cause of stress (Boissy 1995; von Holst 1998). Rabbits released in an unknown area do not know where to feed, to rest or to take refuge against predators. Hence, a possible solution may be to progressively acclimatise the translocated individuals to their new environment. We assessed such a soft release protocol by keeping rabbits in acclimatisation pens that fenced the release warrens during the first few days after translocation, the period during which mortality and movements were known to be the largest. Moreover, acclimatisation was expected to promote the settlement in the release warren, but it was not always the case as some rabbits escaped from the pens or left the warrens after fence removal, possibly due to homing behaviour. In a first experiment, we compared the fate after release of 52 three-acclimatised days individuals and of 52 non-acclimatised ones (study W-h, Table 1; Letty et al. 2000). There was no clear advantage of the acclimatisation procedure on overall survival ($P_{LRT} > 0.7$), despite a significant difference in early survival between sexes in acclimatised individuals: all the females survived, but only 80% of the males. Otherwise, the overall survival was high in this study, maybe due to a good habitat quality. Moreover, the difference in early survival between acclimatised females and males recorded in Letty et al. (2000) was not confirmed in further studies (Letty et al. 2002b, 2005). There was no difference in survival between a 9-acclimatisation day treatment and control treatment in another release (study W-i, Table 1 and Fig. 4). In this case, acclimatisation only delayed predation by raptors or mammalian carnivores, but the final outcomes of the two treatments were equivalent. In another study testing this soft release protocol, Calvete and Estrada (2004) detected a positive effect acclimatisation of, i.e. an increased survival and a decreased dispersal, only in low cover habitat, but not in high cover habitat. Then, the relevance of acclimatisation in rabbit restocking appears to depend on the habitat quality. In general, acclimatisation in animal translocation had either a positive effect (Davis 1983; Bright and Morris 1994; Carbyn et al. 1994) or not (Lemnell and Lindlöf 1982; Fiechter et al. 1988; Benmergui et al. 1990; Castro et al. 1994; Lovegrove 1996). It is likely that acclimatisation does not suppress other negative effects that are very strong, but merely delays them until individuals venture outside after pen removal. A solution might be to extend the acclimatisation period until the onset of the breeding, in order to preserve the breeders from predation and to promote their settlement in the release warrens (Berger et al. 1998). However, such an extended acclimatisation, which begins to resemble rabbit farming, might also add other stresses induced by captivity or social behaviour. Other research studies have examined the use of quarantine in captivity for a few days or weeks before the release in Snowshoe hares and European rabbits, but this protocol generally did not increase restocking

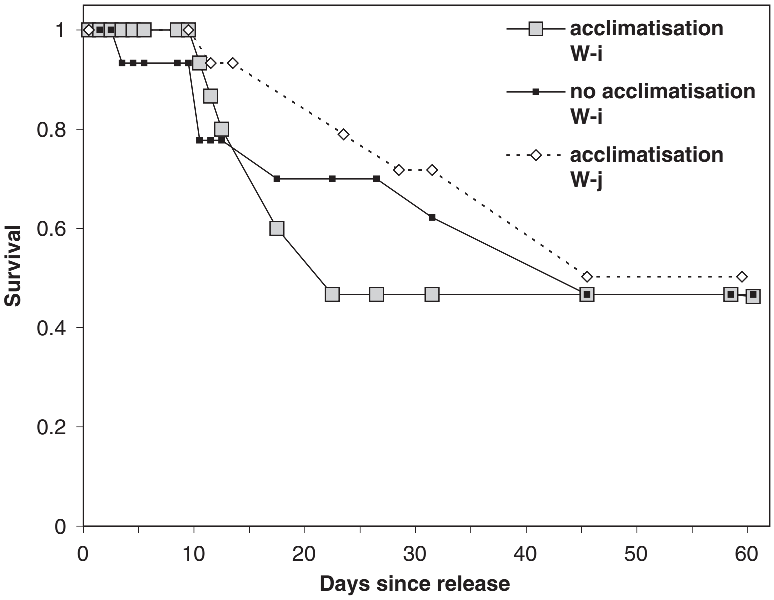

Fig. 4 Survival rate after restocking of translocated rabbits, at least 3 months old, which experienced either acclimatisation or not ($n = 15$ in each batch). Rabbits, translocated from Scotland to Mediterranean scrubland areas in southern France, were released after a 9-day acclimatisation in pens (studies W-i and W-j, Table 1). Survival curves are derived from known-fate data (radio-tracking), and mortality induced by radio-collar and signal losses were both considered as 'losses on capture', and not as real mortality

success, except through the reduction of disease risks (Schultz 1980; Calvete et al. 1997; Calvete and Estrada 2004; Moreno et al. 2004). In particular, Calvete et al. (2005) reported a survival rate of 53% within 22 days after rabbit release. Survival did not depend on the length of the quarantine (2, 4, 6 or 8 weeks).

Beyond the problem of the novelty of the environment, the question of its ecological quality also arises. Obviously, the ecological quality varies among release areas, but it is not easy to determine the habitat characteristics necessary to achieve success in rabbit restocking, or on what ecological factor restocking success depends, and on what spatial scale this factor has its effect. For instance, we have some difficulty in explaining differences in survival or dispersal after release among release areas whose landscapes look alike, and even among neighbouring release warrens, apart from the obvious fact that not all areas and warrens are equally appropriate for rabbit restocking (Letty et al. 1998, 2002a, 2005).

Predation by mammals or raptors seems to be a very important factor for restocking success in European rabbit (Calvete et al. 1997; Letty et al. 2002a; Moreno et al. 2004), as in European hare *Lepus europaeus* (Angelici et al. 2000) and in other prey species (Short et al. 1992), at least at the beginning of the translocation. Indeed individuals are particularly vulnerable to predation when they have just been introduced in the new environment, and some resident predators might take the opportunity to perform surplus killing (Kruuk 1972). Thus, predator control in the release area just before or at the beginning of the translocation might improve early survival and release success. Calvete and Estrada (2004) tested methods to prevent predation by either electric fencing of release warrens or night shooting of predators. They observed that these methods increased short-term survival of rabbits, but did not decrease dispersal, and they recognize that such methods are not always cost-effective or cannot be applied everywhere. In fact, completely effective predator control is difficult to achieve (Stahl and Migot 1993; Mayot et al. 1998), and then, there is often only a temporary positive effect on survival of translocated prey species (Soderquist 1994; Combreau and Smith 1998). The presence of numerous alternative prey does not guarantee a reduced predation rate of the released individuals, as recorded in Mountain hare *Lepus timidus* (Lemnell and Lindlöf 1982), indicating that released individuals are particularly prone to predation. Finally, predation seems to be a serious and difficult problem for translocation of prey species, but it is likely that the recorded impact of predation partly consists, in fact, of predation on dying released individuals or scavenging of those already dead, that could not overcome the difficulties due to the translocation.

The vegetation cover in the release area also affects rabbit restocking success in the short-term, probably through the availability of resting places and of food. For instance, the direction of rabbits' dispersal movements after release was apparently partly determined by the structure of the vegetation cover, with open landscapes preventing dispersal (unpubl. data). In line with this, Calvete and Estrada (2004) recorded both an increased survival and a decreased dispersal after release of rabbits when released in a high cover area (vegetation cover exceeding 75%, height around 75–100 cm), compared to a low cover site (vegetation cover about 25%, height less than 50 cm). Moreno et al. (2004) obtained similar results with rabbits released in a wet scrubland (67% scrub cover, >1.5 m height, fresh herbaceous biomass: 13,025 kg/ha), compared to a dry scrubland (55% scrub cover, <1.5 m height, fresh herbaceous biomass: 5,375 kg/ha). Amongst other lagomorphs, similar results were found in snowshoe hares, in which individuals released in habitat with good cover had a higher survival or smaller movements than those released in poor cover habitat (Sievert and Keith 1985; Wirsing et al. 2002) and also in European hares, in which dispersal after release also seems to depend on vegetation cover characteristics of the habitats (Benmergui et al. 1990; Marboutin et al. 1991).

Moreover, the ecological quality of the release area depends on the season, through the availability of vegetation cover, predation risk, and weather conditions, which could all affect the behaviour and survival of translocated individuals and their subsequent breeding success. Concerning this matter, Moreno et al. (2004) compared the fate of rabbits that were released in the same area but in the four different seasons. Dispersal activity was overall greater in individuals released in autumn or in winter than in those released in summer or in spring, which roughly corresponded to the seasonal differences in movement of resident rabbits. However, survival after release did not differ among seasons of release. We recorded a high survival after release in both juveniles and adults during several summer rabbit translocations, which seems to be at least as high as the survival generally recorded in winter restocking (Fig. 2). In particular, the mortality early after release seems to be less sharp in summer than in winter. Otherwise, dispersal of wild individuals was apparently as high in summer as in winter. In contrast, young European rabbits (at least 3 months old) showed a lower dispersal rate at the beginning followed by a gradually increased dispersal (unpubl. data). However, it is true that these juveniles were born in an extensive breeding enclosure with artificial warrens, which could partly explain their low dispersal (study S-d, Table 1). Our results do not provide clear experimental evidence of the effect of the season of release because they were recorded during several restocking trials that were conducted in different areas and years, and following different protocols (Table 1). Nevertheless, we note that there may not exist a perfect experimental design to test the effect of the season of release because of confounding effects that occur when subsequent batches are released in the same area (effect of the order of the batch), or when they are released in different areas (effect of the area). The season of release seems to be an important factor for restocking success, and we believe that summer may be the best season in western France to release rabbits as the concurrent availability of cover and food likely promotes both reduced dispersal and high survival. Indeed, seasonal variations of food availability in the environment may affect body condition and movement behaviour of translocated animals, and consequently also survival after release (Bright and Morris 1994; Carbyn et al. 1994; Moreno et al. 2004). The good restocking success may also be due to a reduced predation risk in summer, as shown in released snowshoe hares, in which vulnerability to predation was significantly lower in summer relative to other seasons (Wirsing et al. 2002). However, we have to keep in mind that in addition to environmental factors that vary over seasons, this apparent seasonal difference in release success may also be explained by differences in age or social status of individuals among release batches. The rabbits released in summer are generally younger than those released in winter, and they have not yet attained breeding status. It may be that young individuals likely are less disturbed by translocation and better able to adapt to the new situation than adult breeders. As an illustration, we recorded high survival after release in young rabbits born in breeding enclosure (study S-d, Fig. 2). At the very least, survival after release seems to be as high in juveniles as in adults, as seen in another rabbit restocking involving both juveniles and adults (study S-e, Table 1), where survival did not depend on body mass ($P_{LRT} > 0.4$).

And finally, from the point of view of the translocated individuals, the ecological quality of the release habitat probably also depends on the characteristics of their habitat of origin, which would influence their level of pre-adaptation to the release habitat. If there is a poor similarity between capture and release areas, which is equivalent to a sharp change in environmental constraints (landscape, soil, flora, parasites, etc.), difficulties in adaptation may arise. These may not necessarily occur only in the short-term as, for instance, parasites are not evenly distributed over the areas (Grès et al. 2000, 2003) and some pathologies might arise gradually after translocation into a heavily infested area. Nevertheless, phenotypic plasticity may also allow rabbits to fit the new environment, and, considering the wide distribution of the European rabbit throughout the world (Flux 1994), this species of Mediterranean origin seems to have great ability to adapt to new environments. As an illustration, almost 60% of rabbits from sandy areas had prolonged survival after translocation into clayey areas (studies S-f, W-b, and -c partly, Fig. 2). Similarly, rabbits that we translocated from Scotland into a Mediterranean scrubland area in France did not apparently suffer from the subsequent exceptional summer drought and related hot temperatures that occurred in 2003 (study ‘W-i’, Table 1). Indeed, among the 30 rabbits that were released in early March, 13 were still alive on late April, ten from mid-May to early September, and nine at the end of summer. Conversely, however, we recorded very poor survival in individuals translocated from wild populations of the Mediterranean area of France to restock extensive breeding enclosures in the temperate area of western France (unpubl. data), although we could not determine whether it was due to the climatic conditions, to the captivity or to the social competition with resident individuals.

Effect of Social Behaviour on Restocking Success

The rabbits’ social behaviour might also affect restocking success, notably through interactions between released and resident individuals. Indeed, in animal translocations, the presence of resident congeners in the release area prior to the translocation may be an important component of the quality of the release habitat. For instance, resident congeners might attract the translocated individuals and encourage them to settle in the release area (Castro et al. 1994; Soderquist 1994). However, it is difficult to determine if such attraction for resident congeners is linked with social behaviour or with the mere underlying existence of a suitable habitat. On the contrary, the resident congeners might also exclude the translocated individuals from the release area, possibly due to territorial behaviour and the related competition for resources or for mates (Carbyn et al. 1994; Sjöåsen 1997). In Snowshoe hares, the movements of translocated individuals were greater within the range occupied by the species than beyond it, which might be attributable to the presence of resident congeners, but also to a difference in predation risk as during the same time period their survival was smaller within the species range than beyond it (Sievert and

Keith 1985). We have recorded in two different studies that individuals released in second batches had smaller dispersal distances after release than the individuals of first batches that were released one week before, which might be explained by congeners' attraction (Letty et al. 2005). However, the poor restocking success that we have conversely recorded in breeding enclosures suggests that social competition occurs with genuine resident rabbits when population density is high.

Social behaviour among released individuals might also affect rabbit restocking success due to the behavioural preference of European rabbits to live in social groups (Cowan 1987). Indeed, in the case of species with an elaborate social behaviour, the prior social or familiar relationships among the individuals that are released together might influence translocation success. More specifically, conserving these bonds is expected to promote translocation success by increasing survival, settlement, or breeding success (Ylönen et al. 1990). However, such a positive effect of the prior familiarity among released individuals does not systematically occur in animal translocations (Boonstra and Hogg 1988; Armstrong et al. 1994b; Sera and Gaines 1994; Anstee and Armstrong 2001). In two different releases of rabbits, we did not record a clear difference in survival between individuals released in either familiar groups, i.e. the individuals were caught in the same warren of origin or in adjoining warrens, or unfamiliar groups (Letty 1998). In the first release (study W-a1, Table 1), the survival during the first 2 days was not any higher in familiar individuals ($n = 21$) than in unfamiliar ones ($n = 28$), in each sex, respectively ($P_{LRT} > 0.1$). Moreover, distances between resting places of individuals from a given release group did not appear to be shorter in familiar rabbits than in unfamiliar ones, and, for instance, a "familiar" female moved far away from its release warren whereas a male and a female of its group were still alive (unpubl. data). In the second release (study W-g, Table 1), the survival during the first 5 days was better in unfamiliar rabbits ($n = 27$) than in familiar ones ($n = 26$), with no differences between sexes ($P_{LRT} < 0.05$). This unexpected negative effect of familiarity could be explained by some environmental heterogeneity among release warrens: apparent survival was in fact very poor in a single familiar group released a little away from the other groups. Otherwise, the mean early survival rates were high: 94% in unfamiliar rabbits and 59% in familiar ones. In another study, we could monitor by radio-tracking the behaviour of a translocated familiar group of seven rabbits (study S-c, Table 1), captured in the same warren, and then released in another single warren after translocation. The familiar group split after release into two groups, at least. The distance between the two males was larger than mean distances between females, or between females and males, possibly due to behavioural and relatedness differences between sexes (Fig. 5). Throughout this time, all individuals remained alive and within 300 m of the release warren, until a female apparently dispersed 3 months after release and was recovered freshly dead around 800 m away from the release warren 5 months later. This suggests that the members of a

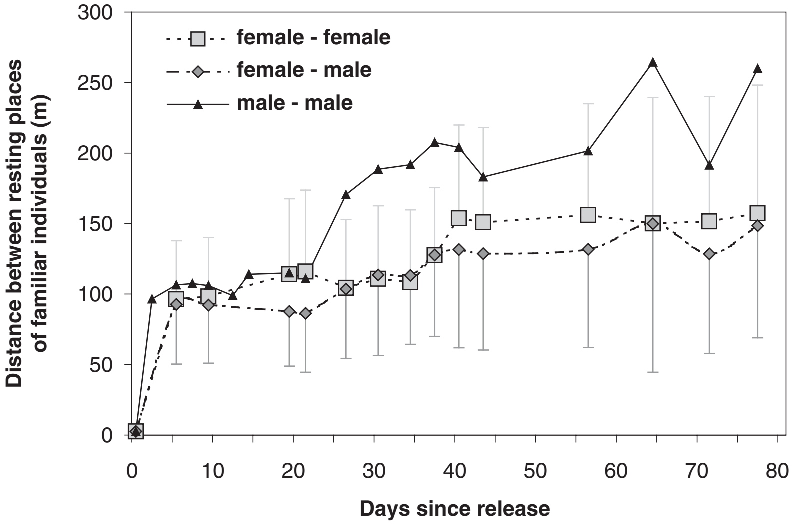

Fig. 5 Mean distance (m, ±SD) between diurnal resting places, in pairs, of rabbits from a translocated familiar group. The release group was constituted of seven familiar adults (5 ♀, 2 ♂), captured before translocation in the same warren, and translocated in the same release warren; all had escaped from the acclimatisation pen 2 days after release. All individuals survived during the first 80 days after release and did not move further than 300 m away from the release warren. Data are split by type of pair of individuals: females, males, or mixed. Radio-tracking data (study S-c, Table 1)

familiar group might not stick together once the territorial behaviour linked to the warren of origin has collapsed. In fact, the disruption due to the translocation event is known to destabilize the prior social relationships in other animal translocations (Armstrong et al. 1994b; Richard-Hansen, Vié and de Thoisy 2000). This may also be the case in rabbits. Moreover, the high mortality and dispersal often undergone by rabbits after release probably does not promote the emergence of a positive familiarity effect. More generally in rabbits, the effect of familiarity within release groups may be not very strong due to the short life span of the species, and the related high natural turnover of individuals within social groups that usually occurs each year. Otherwise, the relationships that underlie familiarity may not be necessarily positive as, for instance, aggressiveness might occur within a social group among dominant and subordinate individuals or among unrelated individuals, and even more among members from adjoining social groups living in the same warren. However, a group of rabbits captured in the same warren may not represent the actual social structure of the rabbit family living in that warren and therefore any effect of "familiarity" in this chapter should be taken with caution and further studies are necessary to test the reliability of this factor.

Conclusions

What lessons about rabbit restocking can we learn from all these field experiments? We first have to keep in mind that the results need to be interpreted cautiously. Indeed, as it is typical for field studies, evidences are not always very conclusive, and the effects of the tested factors (acclimatisation, familiarity, tranquillisation, etc.) may possibly be altered by some untested environmental factors. For example, it is difficult to assess how a treatment factor could improve survival when survival was very high in the control group anyway. Nevertheless, this may indicate that such special treatment is not that important for survival rate in translocated rabbits. It is clear that each experiment should be repeated several times in the field to get significant results, which is unfortunately very expensive and difficult to achieve. However, when comparing the results from all these restocking trials, following a logical and step-by-step approach, we can get general insights into rabbit restocking issues and have some hints of main phenomena.

In European rabbits, as in many other vertebrate species, translocation is a great hardship for the released individuals, and it is a very complex process whose chances of success depend on several factors that act concurrently. Some mortality after release is not surprising during a rabbit restocking, and probably unavoidable since it seems mainly to be induced by the change of area. Moreover, the precise factors determining mortality after release often remain unexplained, and mortality probably results from particular combinations of local environmental components. Nevertheless, it seems possible to promote survival after release, and consequently translocation success, by choosing the best-suited individuals and by a relevant management of the habitat and wildlife in the new environment, before and after the restocking. Finally, a survival rate around 60–70% 2 months after release in a European rabbit restocking must be considered a good result (Fig. 2). Dispersal after release is not a major problem for translocation success since it is generally moderate (less than 300 m). If some dispersal occurs, wildlife managers may compensate by releasing rabbits in neighbouring warrens to ensure there are enough individuals still alive a few months after release and close from each other to provide the foundation of a viable population.

Nevertheless, the subsequent breeding success of the released rabbits that have settled in new areas remains unclear, and it needs to be studied as it is an important part of the overall restocking success (Letty et al. 2002b, 2003b). Little is known about the factors influencing subsequent breeding, and for instance, there are no data concerning the chronology of breeding after release, including the possible waiting period before the onset of breeding in the release area. Moreover, there may be longer-lasting effects on breeding success after translocation than on survival due to the change of some environmental constraints linked to the change of area. Otherwise, we think that releasing young rabbits in summer is a promising solution to increase restocking success, as they are likely to be the individuals best suited to the challenge of translocation, and undoubtedly those with the highest life and breeding expectancies. Therefore, in France, we have encouraged European rabbit farming in breeding enclosures around 1,000–2,000 m^2 to allow small hunting societies to provide themselves with young rabbits to restock their own estates. Indeed, thanks to health measures and high-quality food supply in such breeding facilities, it is possible to produce each year around 100 young European rabbits from about 15 adult breeders, which seems to be cost-effective. But, in this context of rabbit farming, it seems particularly important to provide semi-natural conditions in captivity, such as semi-natural warrens, in order to produce individuals suited to living in the wild. We must also keep in mind that restocking is only one aspect of European rabbit management that hunters and wildlife managers have to carry out. Indeed, in the European countryside that is every day more and more affected by the modern practices of agriculture and other aspects of economic development, the European rabbit will need more and more active involvement from wildlife managers.

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Recovering the Endangered Riparian Brush rabbit (*Sylvilagus bachmani riparius*): Reproduction and Growth in Confinement and Survival after Translocation

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Introduction

The Brush rabbit (*Sylvilagus bachmani*) is distributed from sea level to about 2,200 m along the Pacific Coast of North America from the Colombia River in the north to the southern tip of the Baja California peninsula (Fig. 1). There are 13 described subspecies (Hall 1981). All subspecies occupy dense, shrubby communities, many of which are fire adapted. The Riparian Brush rabbit (*Sylvilagus bachmani riparius*) occupies a range disjunct from other Brush rabbits, near sea level on the floor of the northern San Joaquin Valley, California, USA. Riparian Brush rabbits live both in old-growth riparian forest (primarily valley oak, *Quercus lobata*), and riparian communities dominated by thickets of willows (*Salix* spp.), wild roses (*Rosa* spp.), blackberries (*Rubus* spp.), California grape (*Vitis californica*), and other successional trees and woody plants. When available, they also use dense, tall stands of herbaceous plants adjacent to patches of riparian shrubs or woody vines. Most activity is near the edges of large patches of shrubs or vines (Williams and Basey 1986). These communities in the San Joaquin Valley have been reduced and degraded to less than 1% of their historical extent, primarily by clearing natural vegetation, irrigated cultivation, and impoundment and canalizations of rivers. Consequently, many riparian-dependent species have been jeopardized, including the Riparian Brush rabbit. This rabbit is listed as endangered by California and the US Fish and Wildlife Service (Williams and Basey 1986; US Fish and Wildlife Service 1998, 2000).

Remaining populations of Riparian Brush rabbits exist today in two areas of San Joaquin County: Caswell Memorial State Park (MSP, about 105 ha) along the Stanislaus River, a major tributary of the San Joaquin River, and an estimated 125 ha in several small, isolated and semi-isolated patches along the channels of the San Joaquin River in the southern portion of its delta leading into San Francisco Bay (Williams et al. 2002; Fig. 1).

Annual censuses at Caswell MSP between 1997 and 2003 captured 0 to 12 rabbits. In a 1993 census, we captured 43 rabbits and estimated a population

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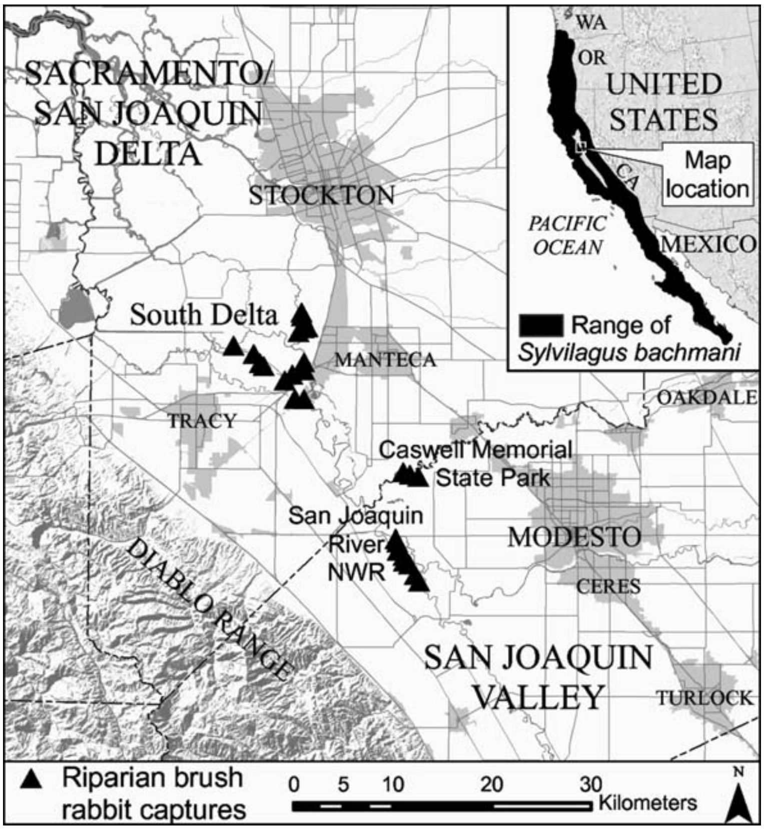

Fig. 1 Distribution map for the Riparian Brush rabbit (*Sylvilagus bachmani riparius*)

size of 67 ± 25.3 rabbits and a density of 3.0 ± 1.13 rabbits/ha (Williams 1993; Williams et al. 2005). Excluding developed areas of the park, Williams (1993) estimated 81 ha with natural communities contained 241 rabbits (approximate 95% confidence interval 170-608). The South Delta metapopulation is larger, probably because of frequent disturbances from farming and flood control that maintains early successional communities. Yet this population is highly fragmented and located entirely on unprotected private properties, many of which are planned for urban developments within 0 to 5 years. Most property owners have allowed access to confirm presence or absence and to capture individuals for captive breeding, but not for estimating population size. We believe that there are at most a few hundred rabbits in the South Delta metapopulation (Williams et al. 2002).

The recovery plan for the Riparian Brush rabbit set a goal of maintaining or establishing three self-sustaining, wild populations outside of Caswell MSP and within the historical range of the species (US Fish and Wildlife Service 1998). To that end, a controlled propagation and reintroduction program was initiated to re-establish populations in suitable historical habitat (Williams et al. 2002) on the San Joaquin River National Wildlife Refuge (NWR) and elsewhere where landowners were willing.

There were between 275 and 325 ha of natural communities on the existing San Joaquin River NWR prior to 1997 (Williams et al. 2002). Following extensive flooding along the San Joaquin River in 1997, the US Fish and Wildlife Service purchased over 530 ha of frequently-flooded private farmland adjacent to the refuge. Levees on the refuge have not been repaired and the former farmland is being actively restored to riparian vegetation. The intent is to allow a return of the normal floodplain and flood dynamics to this area, lowering the flood level so that existing levees could be vegetated and serve as refugia from flooding. Additionally, a mound was constructed next to but higher in elevation than the flood levee bordering the best habitat for Riparian Brush rabbits. The mound is meant to serve as a refuge for rabbits from the highest anticipated flood level. As of 2004, approximately 325 ha of the former farmland had been planted with riparian trees and shrubs. More than 200 ha are scheduled for restoration in 2005 and acquisition of additional property along the river is in progress (Sacramento River Partners 2002).

There has been no information published on survivorship of Brush rabbits. Annual survival of cottontails can vary substantially. For example, annual survival of adult, radio-collared Eastern cottontails (*Sylvilagus floridanus*) was 15% (Trent and Rongstad 1974) and 21% in two areas (Rose 1977).

There has been little information on reproduction reported for Riparian Brush rabbits. Breeding extended from February to May or June for Riparian Brush rabbits in one study (Basey 1990) and from December to May for other brush rabbits in coastal California (Mossman 1955). Breeding by young-of-the-year has not been observed, but breeding by females greater than about 90 days old has been reported for some other *Sylvilagus* species (e.g., Powers and Verts 1971; Chapman et al. 1980).

Based on numbers of embryos, mean litter sizes varied from 2.7 to 3.4 over 2 years in Oregon brush rabbits (Chapman and Harman 1972) and from 3.5 to 4.0 in two areas of coastal California (Orr 1942; Mossman 1955, respectively). Mean annual production of young by female brush rabbits in Oregon was estimated at 15.3 (Chapman and Harman 1972). Mean number of young born typically is fewer than the number of embryos for cottontail rabbits (Chapman et al. 1977).

Herein we report survival, causes of mortality, reproduction, and growth of Riparian Brush rabbits during the first 2 years of operation of the propagation program.

Materials and Methods

Animals selected for breeding were placed in fenced enclosures (0.50–0.57 ha) larger than their typical home ranges (0.33 ha, Dixon et al. 1981). Large patches of Himalayan blackberries (*Rubus discolor*; Fig. 2) and ruderal grasses and forbs provided food and cover within enclosures. No supplemental

Fig. 2 Pen for controlled propagation of Riparian Brush rabbits measuring 162 m long, 30.5 m wide and with side fencing of 2.14 m in height. The top is covered with netting to prevent raptors from entering and the sides are covered with sheet metal (shown on the *left*) but not yet installed on the *right* (photo by L.P. Hamilton)

food was provided. In 2001–2002, only one pen was completed for use. It was populated with six rabbits (3M, 3F) in November–December 2001. These rabbits were initially fitted with radio collars consisting of a cable within Tygon tubing (Holohil); the collars were removed in March because the rabbits were catching their forelegs and jaws under the collar. The pen was emptied of rabbits in November 2002. All three pens were populated with six rabbits each (3M, 3F), newly captured from the wild, in December 2002. We captured breeders from the South Delta, selecting individuals from as far apart as possible to ensure low kinship values. Straight-line distances between capture points of rabbits chosen for captive breeding in 2002 varied from 1.1 to 6.1 km. Distances between capture points of animals selected for breeding in 2003 were shorter, ranging from 0.1 to 4.6 km, but animals of opposite sex placed in the same pen had capture points >0.25 km.

We operated traps in pen 1 at 2-week intervals in 2002, starting 22 February. In 2003, we trapped twice weekly in pen 1 and once weekly in pens 2 and 3. Upon capture, we assessed general health and appearance, visible reproductive condition, weighed adults and offspring, and measured offspring. To calculate gain in mass and estimate age of young rabbits, animals heavier than 600 g were not included because their weights probably were influenced by reproductive condition. To estimate age using mass, we assumed

4-day-olds weighed 28 g (Davis 1936) and used mean gain in mass/day calculated for all young between first capture and last capture when they weighed <600 g. The difference in mass at first capture between years and pens was compared using Student's *t*-test, assuming unequal variance.

Parentage of rabbits born in captivity was determined using eight polymorphic microsatellite loci: (Surridge et al. 1998; Williams et al. 2005). Parental genotypes were determined and compared with the first 15 offspring in 2002 and the first 40 offspring in 2003. Parentage of other offspring was not determined because of problems identifying parents of potential F2 and F3 matings and of backcrosses between generations.

Surviving breeders (3) were removed from the pen in July 2002 and repatriated to their original capture points after attaching a radio transmitter. Additionally, three radio-collared rabbits born in confinement were released at the capture sites of the dead brood stock. These rabbits were monitored by radio-telemetry biweekly through 15 Feb 2003. We were unable to monitor repatriated rabbits in 2003 because of access restrictions on private property.

Young rabbits produced in the pens were released at the San Joaquin River NWR after they had attained adulthood (>500 g). They were fitted with a radio transmitter with a mortality signal and a neoprene-strap collar (ATS model M1750). They were released into soft-release pens (about 0.5 ha in size) that were placed in suitable habitat on the refuge. After acclimation periods of 1–20 days (most were held for 5 days), the pen was opened and the rabbits were allowed to disperse. While in the holding pen, and for 1 week immediately after introduction, the rabbits were monitored daily by radio telemetry. The rest of the year, animals were monitored for status twice weekly. The remains of dead rabbits were located and cause of death was assessed.

Survival of translocated rabbits was calculated from date of introduction to the soft-release pen to date of mortality signal or other evidence of death. For life table analyses of the 2002 cohort, deaths were summed per 28-day period from 31 July 2002 through 12 June 2004 (682 days). For comparisons of survival between years, deaths were grouped in 14-day intervals for rabbits released before November each year and tracked through February of the ensuing year (maximum of 208 days of exposure). Survival and life table analyses were performed on data with censoring using Statistica, version 6.1 (2002). We used Cox's *F*-test to compare survivorship between years.

Results

We captured 340 captive-born young (62 in 2002, 278 in 2003, Fig. 3). Mean mass at first capture was 272.2 g (Table 1) and did not differ by year for pen 1 ($P = 0.79$) or between rabbits in pens 2 and 3 in 2003 ($P = 0.44$), but rabbits in pens 2 and 3 differed significantly from rabbits in pen 1 in 2003 ($P > 0.001$). The first offspring of 2002 were captured on 22–23 February, and weighed 140

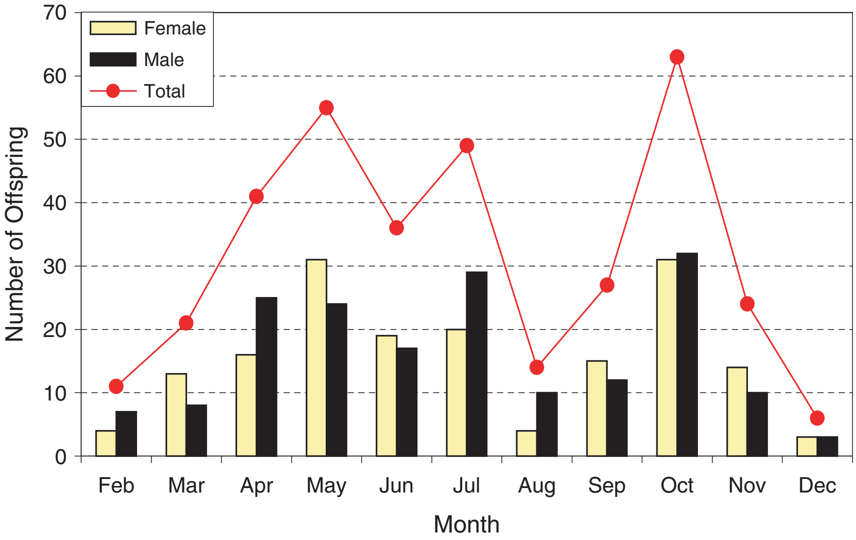

Fig. 3 Number of young rabbits newly trapped and marked in the controlled propagation pens by month in 2002 and 2003 combined

Table 1 Number of young Riparian Brush rabbits captured and mass at first capture in the controlled propagation facility in 2002 and 2003

	n	Mass (g) at first capture $\bar{x}$ (*sd*)	Min	Max
Pen 1 - 2002	62	236.3 (90.17)	110	480
Pen 1 - 2003	111	258.6 (112.71)	70	580
Pen 2 - 2003	80	308.7 (158.22)	105	570
Pen 3 - 2003	87	330.4 (171.84)	80	800
Total	340	272.2 (141.72)	70	800

and 179 g. In 2003 (10–11 Feb), the first-captured offspring weighed 115 and 180 g. The Y-intercept for the regression of change in mass by time for 123 young, non-reproductive rabbits was 21.8 g (Fig. 4). Mean gain in mass was 6.8 (±2.80) g/day for rabbits weighing 70 g or more initially.

The sex ratio was 1.08M:1F (Yates corrected Chi-square 0.39, $P = 0.53$). In 2002, all three of the adult females exhibited evidence of producing young; two had three or four litters each. There was no evidence of females producing more than four litters. Twelve juvenile females reproduced in 2002. Of these, two had two litters. Minimally, there were 22 pregnancies by 15 females producing 62 live young. The mean number of young per pregnancy surviving to a trappable age was 2.8. We removed all the males from the pen that we could

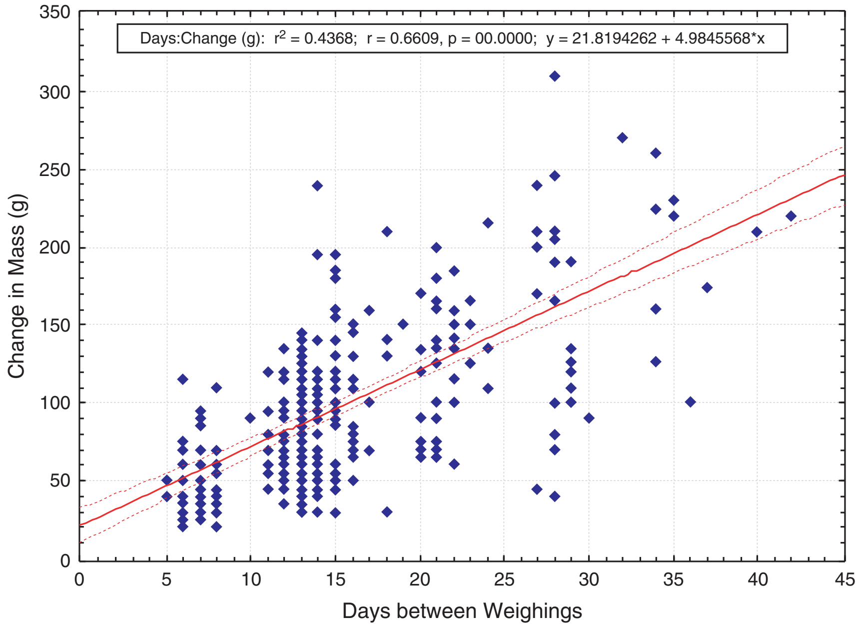

Fig. 4 Scatter plot depicting increase in mass between weighting periods in days for 123 young Riparian Brush rabbits born at the Controlled Propagation facility in 2002–2003

trap and which were of sufficient size to radio-collar, starting on 31 July. We found no evidence of oestrus or pregnancy after 19 September.

In 2003, one female died before breeding and was replaced; nine remaining females reproduced, as did 44 young-of-the-year. We detected 97 pregnancies by 53 females that resulted in 278 young that lived long enough to be trapped. The mean number of young per pregnancy surviving beyond the first few weeks after birth in 2003 was 2.9. There were pregnancies into mid-October and lactation into mid-November. Potential reproduction of offspring was slowed starting 3 July by translocating to the wild those offspring that passed health exams, were not lactating, and weighed >500 g.

All six breeders contributed to producing the first 15 offspring in 2002 based on analyses of parental and offspring genotypes. Seven (of eight) microsatellites were variable in this set of adults. Paternity was not resolved by microsatellites for two young. One male fathered eight of the 15 young while another fathered from four to six, and the third male from one to three. One female produced six young, another produced eight, and the third, which died of a collar-related accident on 1 March, produced only one. In 2003, parentage was determined for 33 and unresolved for seven young born early in the year. In pen 1, where more genotypes were analyzed, a single male dominated breeding (13 young from three females), but one other male also mated with all three females (5–6 young), whereas the third male mated with only two females, producing 2–3 offspring. The three females produced 11, 4–6, and 4–6 young. Similar patterns of parentage were seen in the other pens although 1 male did not produce any of the young we tested.

Forty-nine of the rabbits born in captivity in 2002 were released at the San Joaquin River National Wildlife Refuge (SJRNWR) between 31 July and 31 October 2002. Three others were released in the South Delta to replace adult breeders that died in captivity. For the cohort raised in 2003, 194 had been released on the refuge by 1 March 2004; of these, 156 had been released between 3 July and 31 October 2003, a period comparable to 2002.

In 2002, three of the six breeders survived over 7 months in the pen (196 days) and then were repatriated to their original capture sites in July. One repatriated rabbit left his original capture site and was located ~270 m and ~545 m away on two different dates. The next time we located him, he had returned to his capture site, all within 1 month of release. On subsequent sessions we could not pick up his radio signal. The other two were alive until we lost contact with one on 20 January 2003 and the other on 19 March 2003. Of three young released at the sites of capture of the adult breeders that died while in the pens, we lost contact with one immediately, and within 1 month contact with the other two was lost.

In 2003, 13 of 20 adult breeders died while in the pens (35% survival, 272 days maximum exposure). The seven that were repatriated to their original capture sites were not monitored, and no young were released to replace the ones that died in captivity.

Survival statistics of captive-born rabbits while in the breeding pens and after release to the wild are listed in Table 2. Young unaccounted for in Table 2 in 2003 were still in the breeding pens at the cut-off date for this report. The percentage of marked young surviving while in captivity in 2003 compared to 2002 was not significantly different (Yates corrected Chi-square, 1.21, $P = 0.272$). Survival during comparable at-risk intervals from release (Fig. 5) was significantly higher for the 2002 cohort compared to the 2003 cohort (Cox's F-test, $F = 1.908$, $P = 0.046$). By 1 March 2003, 30 of 49 rabbits translocated

Table 2 Survival rates of Riparian Brush rabbits involved in controlled propagation and translocation to the San Joaquin River National Wildlife Refuge

Captive born rabbits	2002	2003
Number	62	278
Died in pen	13	40
Survival rate in captivity (days exposed[a])	0.79 (298)	0.86 (450)
Number translocated	49	196[b]
Died after release	23	152[b]
Survival rate after release (days exposed[a])	0.23 (673)	0.22 (317)[b]

[a] Indicates the maximum days of exposure for an event

[b] All animals translocated from July 2003 through February 2004

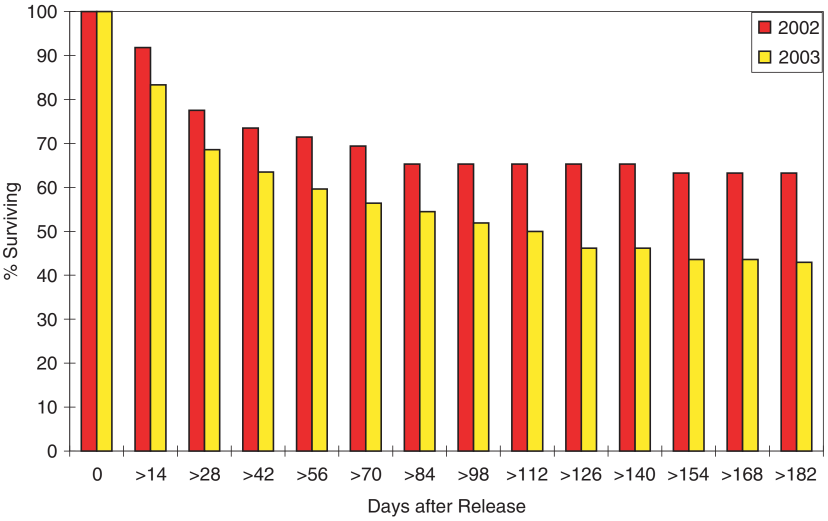

Fig. 5 Survival of two cohorts of captive-breed Riparian Brush rabbits released on the San Joaquin River National Wildlife Refuge in 2002 and 2003. Data for each group span a period from July through February of the ensuing year

in 2002 were alive (61% survival), and of the 2003 cohort (156), 69 were alive on 1 March 2004 (44% survival).

After 682 days of monitoring the 2002 translocation cohort, five rabbits were alive, we had lost radio contract with 18, and 26 were known to have died. The cumulative survival rate for this period was 23.1% (±7.6%). Median life expectancy of 376 days peaked at 99 days (SE>97.9 days) after release. The survival rate 1 year after release was 49% (±7.6%) for the 2002 cohort, based on life table analysis.

Cause of mortality (Table 3) was unknown for the largest number of deaths. In the propagation pens most deaths due to unknown causes were of rabbits that were not recaptured and no remains were found (these animals were not radio-collared). Likewise, for translocated rabbits, either no remains were found (except the radio-collar) or the remains were too few to determine cause. Where disease was determined to be the likely cause, *Baylisascaris* sp. was most often implicated. Other diseases implicated in deaths were necrotizing typhlitis, and intestinal lymphoma. Predation, including presumptive predation, was the greatest cause of deaths in translocated rabbits. We noted problems with loss of collars and catching legs or jaws in cable-tubing-type collars, either because of improper crimping of the cable or inability of some crimps to hold under the force exerted by the forefeet of the rabbits. One adult breeder died by catching its lower jaw under the cable of the collar. After this incident, we removed the collars from the confined rabbits. All the translocated rabbits had strap collars, which were the source of several non-lethal injuries as well as one known mortality.

Table 3 Number of mortalities by cause for Riparian Brush rabbits in the controlled propagation and translocation program

	Predation			Accident				
Group	Mammal	Avian	Probable	Collar	Trap	Disease	Newborn	Unknown
Propagation Pens								
2002	0	0	0	1	1	3	1	8
2003	0	0	1	0	5	16	7	11
Translocation								
2002	1	1	9	1	0	0	0	20
2003	9	9	22	0	0	4	0	59

Discussion

In both years of captive propagation, reproduction of Riparian Brush rabbits started earlier (December versus February) and extended much later (October versus May; Mossman 1955; Williams 1988; Basey 1990) than had been seen in the wild for this or other subspecies. For the breeding season, successful production of young per breeding female (5.3) was much lower than the production of 15.2 newborns per female estimated by Chapman and Harman (1972) based on number of embryos. Our productivity estimates are for young living to independence or beyond. Further, the successful productivity per female included substantial contributions from rabbits born earlier in the same breeding season, something that neither Mossman (1955) nor Chapman and Harman (1972) observed in the wild. Three factors could have led to lower productivity than the theoretical estimates for adult breeders: having fewer live young per litter than the mean number estimated by counting embryos; having one or more young, on average, die before reaching a trappable age; and having fewer than expected litters. We have not tried to determine the numerical contributions of each factor, but only about a third of the brood-stock females had more than one or two litters instead of the four to five predicted by Chapman and Harman (1972). Yet, the realized production of rabbits living to be translocated from the three pens was between two and three times greater than the minimum we had estimated *a priori* (Williams et al. 2002), due mostly to the reproduction of young females whose contribution was unexpected.

That mean mass at first capture was significantly greater in pens 2 and 3 compared to pen 1 probably is explained by the less frequent trapping in pens 2 and 3. Our understanding of growth of young and the calculated relationship between age and mass suffers from lack of data on the change in mass between birth and weaning, data that could not be obtained because nests were inaccessible in large clumps of blackberry vines. We recognize that growth is not linear and our method probably underestimates age by a few days. We estimated the smallest, live-trapped individual (70 g) to be about 10 days old—eastern cottontails were thought to be capable of leaving the nest when 12–16 days old (Chapman et al. 1980). Mean estimated age at first capture of all young was 40 days. Young at weaning weighed about 96–103 g, assuming that weaning was at about 14–15 days of age, based on other *Sylvilagus* species (Chapman 1974, 1975; Chapman and Wilner 1978). Young Riparian Brush rabbits attained reproductive maturity when about 77–84 days old (550–600 g).

Based on the results of geneotyping, Riparian Brush rabbits in the controlled propagation pens exhibited a polygynous mating system, with one male dominating mating of females but not to the exclusion of all other males. Females exhibited promiscuity in that some litters were fathered by more than one male.

The relatively large number of translocated rabbits with which we lost contact probably had dead transmitter batteries. Our resources were not sufficient to locate and trap all rabbits and replace failing batteries because of the large area over which they had dispersed. Mortalities attributed to radio-collars (two of 286, Table 3) were few, but are major concerns in any study (Bond et al. 2000). More deaths than we detected may have been associated with radio-collars because of neck abrasions and other injuries from radio-collars that were found on several rabbits when trapped. Strap-type radio-collars eliminated most accidents with legs or jaws becoming caught under the collar, but were a worrisome cause of abrasions and wounds. Trap accidents included two rabbits being caught simultaneously in the same trap, with subsequent injuries from fighting, broken cervical vertebrae from collision within the trap, and accidentally leaving a trap set when unattended. All were exceedingly rare considering the number of times rabbits were trapped.

Repatriation of the wild-caught brood stock after 7 months in captivity was a limited success (2 of 3 surviving from July to beyond January), but releasing captive-reared young at the capture site of their dead progenitors was not successful the first year and was not done the second year for logistical reasons. We believe it is important in situations such as this that the captive-propagation program not significantly impact the natural populations that are the sources of breeders (Williams et al. 2002). Unfortunately, access and activity restrictions on private property precluded us from measuring possible impacts.

There was no evidence from health exams, which included blood chemistry for several captive rabbits, or from necropsy of rabbits that died in captivity, that any rabbits were undernourished or malnourished (Williams et al. 2005). We also saw no evidence that vegetation within the propagation pens was being over-browsed or otherwise adversely impacted by rabbits. We conclude that providing adequate space and abundant natural, live vegetation for rearing brush rabbits were successful in avoiding potential problems of husbandry in captivity related to diet.

That 61% and 44% of the translocated rabbits survived from release through February of the ensuing year, when the females could have had two or more litters, exceeded our expectation of less than 20% survival through what we predicted would be the breeding season (Williams et al. 2002). That survival was so high for the 2002 cohort may have been due to an absence of predators cued into brush rabbits at the release site. Perhaps survival was significantly lower for the second (2003) cohort because more predators had been attracted by the population of rabbits established in 2002. Some females in the cohort translocated in 2002 were pregnant when translocated, others bred soon after, and survivors and offspring bred during the next breeding season. These rabbits may have occupied much of the most suitable habitat, forcing more rabbits released in 2003 into poorer-quality habitat where they were more vulnerable to predation. This suggests that perhaps the best conservation strategy for repopulating unoccupied habitat is to make the largest and most genetically diverse release the first or only release instead of releasing smaller numbers in two or more years. We plan to use and assess different reintroduction strategies on other properties that are expected to become available for repopulation of Riparian Brush rabbits, and compare them with the population on the San Joaquin River NWR. When sufficient data are available, we also will compare survival of rabbits held for various periods in the soft-release enclosures.

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Conservation of Critically Endangered Lagomorphs: The Tehuantepec Jackrabbit (*Lepus flavigularis*) as an Example

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and Consuelo Lorenzo[3]

Introduction

The Tehuantepec jackrabbit (*Lepus flavigularis*) is a Mexican endemic lagomorph species that is critically endangered by habitat loss and fragmentation, overhunting, small population size, and genetic isolation. As the most endangered among five species of jackrabbits in México and, in fact, one of the most endangered mammals in the world (Flux and Angermann 1990), the Tehuantepec jackrabbit is protected by the Mexican Official Norm on endangered wildlife and the IUCN Red List of Endangered Species (although jackrabbit habitat is not protected). The total species population size is probably less than 1,000 and divided into three isolated subpopulations that occur along the shores of Superior and Inferior Lagoons in the Isthmus of Tehuantepec, Oaxaca, México (Fig. 1). Conservation laws are not enforced and jackrabbits are hunted for sport or subsistence. In addition, jackrabbit habitat is used as cattle pasture and is deteriorated as the result of introduced exotic grasses, induced fires, agriculture, and human settlements.

Previous studies on Tehuantepec jackrabbits have included cerotype analysis, histology of ovaries and vagina, and phylogenetics (Lorenzo 1996; Portales 1996; Cervantes and Lorenzo 1997). More recently, surveys have showed that jackrabbits were found in well-conserved grassy plains and coastal dunes, but were not observed in agricultural lands, overgrazed grasslands, or continuous dense vegetation (Vargas 2000; Sántis 2002). In addition, Farías (2004) conducted radio-telemetry research ($n = 51$ individual jackrabbits) to specifically assess home range size and overlap to gain insight into the jackrabbit's social organization, habitat use to identify vegetation that should be target of conservation efforts, and cause-specific mortality factors that threaten survival of jackrabbits. One of the consequences of this work was the ability to better assess conservation needs of the species.

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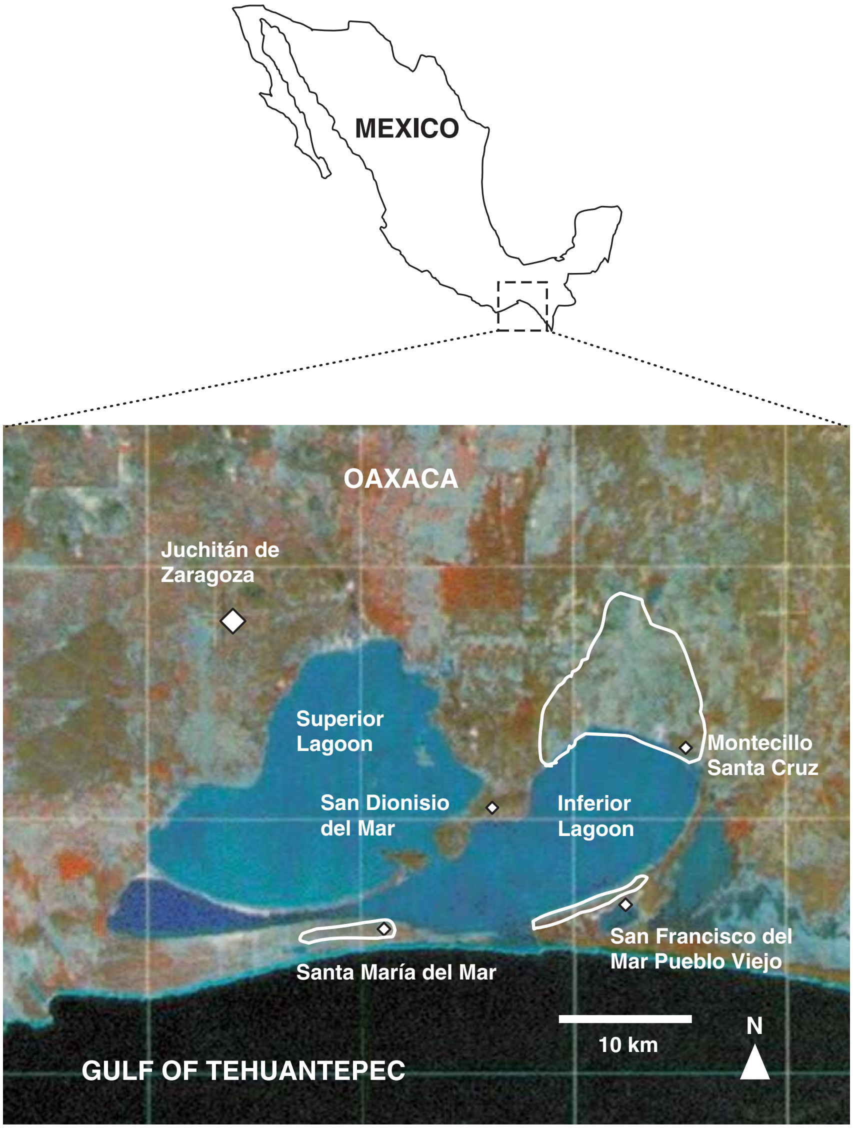

Fig. 1 Estimated extent of occurrence for three remnant populations of Tehuantepec jackrabbit (*Lepus flavigularis*) in Oaxaca, México, is indicated with *white circled areas*

Geographical Distribution

The former range of the Tehuantepec jackrabbit is not documented in detail, but the geographic distribution of *L. flavigularis* is entirely tropical and represents the southernmost limit of the genus in North America (Hall 1981). The species was named after its geographic range along the Mexican Pacific coast on the Isthmus of Tehuantepec, from Salina Cruz in Oaxaca to Tonalá in Chiapas (Nelson 1909), an area of perhaps only 5,000 km^2. The present extent of occurrence was estimated to be about 520 km^2 for three remnant populations found (1) on the sand bar of Santa María del Mar, (2) on the sand bar of San Francisco del Mar Pueblo Viejo, and (3) in a tropical dry savanna between San Dionisio del Mar and Montecillo Santa Cruz (Fig. 1).

Farías (2004) studied Tehuantepec jackrabbits in a savanna 2 km northwest of Montecillo Santa Cruz, an area typical of jackrabbit habitat. Native vegetation in this area includes grassy plains dominated by grama (*Bouteloua* spp.) and paspalum (*Paspalum* spp.), open shrubbery dominated by nanche (*Byrsonima crassifolia*), and scattered trees dominated by morro (*Crescentia* spp.; Pérez-García et al. 2001). Local people practice subsistence fishing and hunting and raise free-ranging cattle, horses, sheep, and goats. Climate is tropical with mean annual temperature of 25°C, mean annual rainfall of 800 mm (García 1964), and marked seasons. Rains occur from May to October with an intra-estival drought in August, and rains stop from November to April with severe drought during late winter and early spring (Zizumbo and Colunga 1982). Native mammals associated with Tehuantepec jackrabbits are Eastern cottontails (*Sylvilagus floridanus*), Nine-banded armadillos (*Dasypus novemcinctus*), skunks (*Mephitis macroura, Conepatus leuconotus*), Southern opossums (*Didelphis marsupialis*), Mouse opossums (*Marmosa canescens*), Gray foxes (*Urocyon cinereoargenteus*), Northern raccoons (*Procyon lotor*), and Coyotes (*Canis latrans*). Gray foxes are potential native predators of Tehuantepec jackrabbits (Trapp and Hallberg 1975) and a Coyote was observed carrying a killed Tehuantepec jackrabbit during a survey (Enrique Martínez-Meyer, pers. comm.).

Conservation Ecology

Farías et al. (2006) found that average seasonal home ranges (95% fixed kernel) and core area sizes (50% fixed kernel with least-squares cross-validation) for adult jackrabbits were similar to annual ranges, did not vary much by season, and were similar for females (58 and 9 ha, respectively), and males (66 and 10 ha). Seasonally, adult jackrabbit ranges overlapped with at least one and up to ten individuals, with mean overlap index of 0.21 for 75 observed dyads over the four seasons of radio-tracking (Farías et al. 2006). Male-male overlap (0.08) was significantly less than female-female overlap (0.26) and female-male overlap (0.23).

Tehuantepec jackrabbits showed significant habitat selection for grassy plains with open shrubbery and scattered trees and avoided dense vegetation to establish home ranges (Farías 2004). Within home ranges, Tehuantepec jackrabbits favored grassy habitats with woody cover but underused grasslands without woody cover. Only 6% of available habitat was grassland without woody cover, but 30% of mortality sites from predated adults were found in this habitat type. In contrast, grassy plains with scattered trees (*Crescentia* spp.)

occupied 36% of available habitat and 20% of mortality sites from predated adults fell in this habitat type. Jackrabbits left their forms at dusk to forage throughout the night and at dawn returned to rest in their forms (Farías 2004). During dusk, night, and dawn, jackrabbits favored grassy habitats with scattered trees (*Crescentia* spp.) for foraging. During diurnal hours, jackrabbits favored nanche (*B. crassifolia*) shrubbery to rest.

Annual survival rates for adult Tehuantepec jackrabbits were 0.51 for females ($n = 13$) and 0.36 for males ($n = 12$), and averaged 0.43 (Farías 2004). Adult females survived better during dry season (1.00, $n = 13$) when compared to wet season (0.53, $n = 13$), but adult males did not (0.61 $n = 11$ vs. 0.59, $n = 12$). Juvenile survival was low, and males survived better than females during wet (0.48, $n = 6$ vs. 0.15, $n = 12$) and dry (0.35, $n = 10$ vs. 0.06, $n = 14$) seasons. Predation was responsible for 67% of adult and 94% of juvenile mortalities (Farías 2004). Human-induced fires caused 20% of adult mortalities, and poaching accounted for 13% of adult and 6% of juvenile deaths.

Discussion

Home range and overlap analysis indicates that differences in spatial behavior may exist between females and males of Tehuantepec jackrabbit (Powell 2000; Farías 2004) and that there is a real need for long-term ecological research. Apparently, Tehuantepec jackrabbits have non-territorial social organization and polygamous mating behavior (Flux 1981; Farías 2004), and establish their home ranges in selected habitats.

Preservation of flora diversity and structure of native vegetation in jackrabbit habitat is urgently needed for conservation of Tehuantepec jackrabbits (Farías 2004). Grassy plains with open shrubbery and scattered trees provide jackrabbits with places to rest and feed, and allow predator detection and attack evasion (Lechleitner 1958). Introduction of exotic grasses and induced fires are activities related to cattle raising that deteriorate jackrabbit habitat (Pérez-García et al. 2001) and may threaten survival of Tehuantepec jackrabbit populations.

Predation was the main mortality cause for radio-tracked Tehuantepec jackrabbits, and juvenile survival was low due to predation (Farías 2004). Juvenile jackrabbits are more vulnerable to predation than adults (Rohner and Krebs 1996), and jackrabbits too young to outrun predators rely on concealment as behavior to avoid being detected (Aanes and Andersen 1996). Induced fires turn jackrabbit habitat into bare areas where jackrabbits may be completely exposed to predators (Farías 2004). Coyotes and Gray foxes, native predators of jackrabbits, may be favored in human-altered and deteriorated habitats (Trapp and Hallberg 1975). Research targeted to identify mortality caused by specific predators, and studies on predator densities are needed for development of potential predator management plans.

Unfortunately, functional programs on predator control and designation of protected natural areas for conservation of Tehuantepec jackrabbits may take several years. However, management strategies to reduce jackrabbit mortalities due to human activities may be developed in the short term if local people integrate into conservation efforts. Adult deaths by fires and poaching added to high predation rates on juveniles threaten survival of Tehuantepec jackrabbit populations (Farías 2004). Until predator control is implemented, poaching and induced fires are human activities that should be controlled.

Educational programs need to be implemented and aimed to facilitate acceptance and involvement of local people into wildlife conservation in their homelands. Cattle raising activities could be oriented for wildlife conservation through active participation of local people. For example, cattle-enclosures discourage non-local hunters from entering jackrabbit habitat because hunters do not trespass cow corrals to avoid confrontation with local ranchers. Cattle densities in jackrabbit habitat could be gradually reduced to a minimum that would guarantee poaching inhibition, and induced-fires could be eradicated.

Strategies should allow local people to gradually abandon cattle-raising activities and instead benefit from wildlife conservation activities. For example, local people that protect jackrabbit habitat could benefit from exclusive use of food, building materials, and fuel in protected land. Until the designation of a natural protected area for Tehuantepec jackrabbits is achieved, jackrabbit habitat could be protected by government agencies and non-government organizations that would pay rent or rights of land use. Nevertheless, economic resources should benefit local communities.

Tehuantepec jackrabbits face continuous habitat loss and fragmentation, small population size with isolated subpopulations, high predation rates, and low juvenile survival. Ecology research on demographic parameters should continue for monitoring of birth ratios, age structure, age- and sex-specific reproduction and survival rates, and specific-predator mortality rates. Estimates of age of first reproduction, pregnancy rates, litter size, and number of litters produced per female per year could be obtained through captive or semi-captive breeding of Tehuantepec jackrabbits. Robust estimates of demographic parameters are needed to develop models on population dynamics of Tehuantepec jackrabbit for conservation efforts.

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A Review of the Biology and Conservation of the Amami Rabbit (*Pentalagus furnessi*)

FUMIO YAMADA

Introduction

The Amami rabbit (*Pentalagus furnessi*, Stone 1900) is a monotpypic and unique ancestral rabbit found in the subtropical and tropical zone of southern Japan (Corbet 1983; Yamada and Cervantes 2005). The rabbit is endemic to Amami-Ohshima (712 km^2) and Tokuno-Shima (248 km^2) islands which originally lacked predatory mammals, in the Ryukyu Archipelago. Both islands are located in the subtropical zone (mean annual temperature of 21°C) with much rainfall (3,000 mm per year). *P. furnessi* is mainly found in mountainous habitats with a forest cover of 86% on Amami-Ohshima Island, and 44% on Tokuno-Shima Island. It occurs in coastal scarp rocks with cycads and mountain forest with oaks. It ranges from sea level to 694 m on Amami-Ohshima Island, and to 645 m on Tokuno-Shima Island, an altitudinal rage low in comparison with other rabbits found in the tropics. Some biological knowledge on the rabbit has been revealed gradually, however, population size of the rabbit has been decreased rapidly, because of human and invasive species impacts. I reviewed biology and conservation status of the rabbit by surveying recent research data in order to understand the rabbit.

Description

Pentalagus furnessi is a medium-sized rabbit clearly distinguished from other genera by several external and cranial characteristics (Fig. 1). Its fur is thick and woolly, dark brown above, becoming more reddish-brown on sides. Its underparts are light reddish-brown and its hind feet, tail, and ears are short, and its eyes are small. The heavy, curved claws are very large and strong (10–20 mm in length), nearly straight on the fore feet, while curved on the hind feet (Stone 1900). Mean external measurements (mm), respectively for four male adults and three female adults, from Kawauchi, Sumiyo, Amami-Ohshima Island, are (range in parenthesis): total length, 451 (430–470), 452 (397–530); length of ear, 44 (40–50), 45 (42–49); length of hind foot, 86

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Fig. 1 Adult (sex unknown) *Pentalagus furnessi* at Sumiyo, Amami-Ohshima Island. Used with permission of the photographer H. Katsu

(80–92), 89 (83–92); length of tail, 27 (20–35), 30 (25–33); body mass (in g), 2,226 (2,030–2,675), 2,477 (2,000–2,880); measurements of one male adult from Mt. Amagi, Amagi, Tokuno-Shima Island, were: total length, 470; length of ear, 44; length of hind foot, 85; length of tail, 25; body mass (in g), 2,240 (Yamada, unpublished).

The skull is low and flat and broad between the orbits, and the rostrum is shorter and heavier than in the other Leporidae (Lyon 1904). Anterior projection of superorbital process is absent as in *Caprolagus*, *Pronolagus* and *Romerolagus*, but posterior projection of superorbital process in *Pentalagus* is well developed, heavier, and more blunt than those in genera. Incisive foramina are narrow, their sides approximately parallel, resembling those in *Pronolagus* in shape, but very much smaller. Audital bullae are very small, being even more reduced in size than they are in *Pronolagus*. Nasals are very short and broad, as wide in front as behind. Sutures of the interparietal are obliterated. The bony plate is long. Zygoma is moderately heavy, its posterior free extremity is moderately long, its antero-inferior angle slightly enlarged but considerably flared outward. Mandible has a very large, rounded angular process, which is separated from the condyle by a small, shallow notch (Lyon 1904).

M^3 is sometimes absent in *Pentalagus* (Lyon 1904; Abe 1931; Hayashida et al. 1967; Corbet 1983). Most genera in the Leporidae are classified based mainly on the number of grooves on the anterior surface of P^2 (three grooves in *Pentalagus* as well as in other genera, while two grooves in *Brachylagus*, *Nesolagus* and *Romerolagus*), the absence (*Romerolagus*) or presence (other genera) of the anterior reentrant angle, the absence (*Lepus, Oryctolagus* and *Sylvilagus*) or presence (*Pentalagus* and *Pronolagus*) of anterointernal reentrant angle, and the morphology (like in *Romerolagus*, connected with posteroexternal reentrant angle in *Lepus, Oryctolagus* and *Sylvilagus*, and reentrant angle in *Pentalagus* and *Pronolagus*) of the enamel at the position of the posterointernal reentrant angle of P_3. Thus, only *Pentalagus* has all five reentrant angles and it has the most complicated enamel crenulation in the Leporidae (Hibbard 1963; Dawson 1958; Tomida and Jin 2002). Radius and ulna are short, the bones heavy, and the radius is distinctly shorter than the humerus; its hind foot is short and stout, tarsal bones are relatively wider than in other genera, and metatarsals are especially short and heavy and their basal width is the broadest in the Leporidae (Lyon 1904). Transverse processes of lumbar vertebrae are the broadest in the Leporidae (Corbet 1983). Apparently, *P. furnessi* has the most primitive characteristics of all the Leporidae (Corbet 1983). The type specimen described by Lyon (1904) is on deposit at the Philadelphia Academy of Natural Science as ANSP#20645.

Evolution of *Pentalagus*

As for the fossil record of *Pentalagus*, two upper molariform teeth (right M^1 and left P^3) of the Amami rabbit were reported from Tokuno-Shima Island as the first fossil record (Tomida and Otsuka 1993). Their geologic age is likely to be late Pleistocene. They occurred with the extinct deer *Cervus astylodon*, suggesting that the Amami rabbit might have arrived at the island at about the same time and through the same migration route as *C. astylodon* during the early Middle Pleistocene. *Pliopentalagus* is a fossil rabbit that is supposed to be ancestral to the living Amami rabbit (Dawson 1958; Hibbard 1963; Tomida 1997). New records of *Pliopentalagus* were found in Huainan, Anhui Province, China (Tomida and Jin 2002), and in Moldavia and Slovakia in Europe (Gureev 1964; Daxner and Fejfar 1967). The fossil materials from China are from the latest Miocene (ca. 6 million years ago) to late Pliocene (ca. 3 million years ago) and are expected to make possible the tracing of the evolution of the *Pliopentalagus* – *Pentalagus* lineage for the last ca. 6 million years (Tomida and Jin 2002).

A phylogeny of the molecular data shows that *P. furnessi* appeared during the first generic radiation of the leporids, in the Middle Miocene, as did another six genera, *Brachylagus, Bunolagus, Lepus, Oryctolagus, Romelolagus* and *Sylvilagus,* and with the exception of two genera, *Nesolagus* and *Pronolagus* which radiated a little earlier (Yamada et al. 2002). The molecular data also suggest that there is no phylogenetic relationship between *Pentalagus* and *Pronolagus*, which had been considered to share a common ancestor (Yamada et al. 2002; Matthee et al. 2004). The phylogenetic tree containing *P. furnessi* had short internal branches and no clustered genera with strong bootstrap relationships (Yamada et al. 2002), which indicates that most of the leporid genera arose during a rapid divergence event (Halanych and Robinson 1999).

Pentalagus was thought to have been remained in the two small islands until now, which has moved from Asia because no predatory mammals such as weasel lived together, except for the crotalid snake, *Trimeresurus flavoviridis* on the two islands.

Adaptation to Damp Subtropical Forest Habitat

The Amami rabbit lives in a damp subtropical climate and its home range size (1.3 ha for four males and 1.0 ha for three females) was much smaller than those of other leporids (Table 1; Yamada et al. 2000). The home ranges of the females do not overlap with each other, whereas those of males do overlap, even with those of females. The data suggest that the mating system of the Amami rabbit is promiscuous like that of other leporids. According to our radio-transmitters, the Amami rabbit is active mainly at night, moving for feeding and dropping their pellets in open places, such as forest roads where food plants are rich, 100–200 m away from their burrows, which are usually located in small valleys covered by dense forests. They frequently use runways to climb up and down steep slopes from burrows, and through mountain streams and undergrowth to open areas. There is an observation of a rabbit swimming down a stream (Kirino 1977).

The Amami rabbit feeds on more than 29 species of plants, including 12 species of herbaceous plants, *Adenostemma lavenia, Carex* sp., *Miscanthus sinensis, Peucedanum japonicum, Mosla dianthera,* etc., and 17 of species shrub plants, *Castanopsis sieboldii, Melastoma candidum, Rubus sieboldii, Styrax japonica, Zanthoxylum ailanthoides*, etc., at Kawauchi, Sumiyo in Amami-Ohshima Island (Yamada, unpublished). According to my observation, stems and twigs smaller than 10 mm in diameter of herbaceous plants and those smaller than 7 mm in diameter of shrub plants were clipped and eaten by the rabbit, and the bark of stems and twigs bigger than 7 mm in diameter of shrub plants was also eaten. Acorns of *Castanopsis seiboldii* that have fallen on the ground during autumn and winter are also eaten by the rabbit. Therefore, the rabbit eats mainly sprouts and young parts of plants, including cambium, and nuts from a wide range of plant species. Food of grasses and young parts of plants are supplied mainly at edge of dense forest or gap.

Table 1 Comparison of home range size between Amami rabbit and other some leporids

Species		Range size (ha)		Body weight (g)		
Common name	Scientific name	Male	Female	Male	Female	References
Amami rabbit	*Pentalagus furnessi*	1.3	1.2	2,226	2,550	Yamada et al.(2000)
Hispid hare	*Caprolagus hispidus*	8.2	2.8	2,248 (1,810–2,610)	2,518 (1,885–3,210)	Bell et al. (1991)
Riverine rabbit	*Bunolagus monticularis*	20.9 ± 2.1	12.9 ± 5.6	2,000–3,000	2,000–3,000	Duthie and Robinson (1989)
Hare and jackrabbit	*Lepus*	10–300		2,000–5,000		Flux and Angerman (1990)

The entrance to the burrow of *P. furnessi* is not often hidden in vegetation because there is very little undergrowth in dense forest. Entrances are horizontal or slightly oblique and of round shape, ranging between 10 and 20 cm high and 12–25 cm wide (Abe 1963; Wildlife Conservation Group 1984). Tunnels follow a straight line for 30–200 cm. L-shaped tunnels follow a straight line 30–200 cm long from the entrance and then are bent at up to a right angle before following a straight line 60–185 cm long to a chamber (ex. 20 cm in diameter) laid with leaves (ex. 6 cm deep). Rabbits also use bases of trees, bases of rocks and the inside of fallen trees for burrows. The entrance 15 cm high and 20 cm wide in the base of a tree is slightly oblique downward and the tunnel follows a gentle horizontal curve 80 cm long (Abe 1963).

The maximum temperatures (21 and 24°C) of the burrows, measured at 0.7 and 1.5 m from the entrances, were 6°C lower than those (27 and 30°C) outside in the daytime in summer (Yamada, unpublished). Rabbits seemed to leave their burrows when the temperature was below 24–25°C at night to avoid heat stress during activity.

The Amami rabbit has quite a unique vocalization, like the pikas (*Ochotona*), and beats the ground with the hind limbs to communicate with others and threaten intruders (Yamada, unpublished). At dusk, rabbits appear at the entrances of their burrows before they become active, and make calls that can be heard loud and clear in small valleys. A mother also vocalizes to attract its offspring when it approaches its nursing burrow. Vocalizations and beating the ground are also produced when humans enter its habitat, not only as a threat call, but to warn other rabbits. Other leporids, such as *Pygmy rabbit (Brachylagus idahoensis)* (Green and Flinders 1980), *Volcano rabbit (Romerolagus diazi)* (Cervantes et al. 1990) and *Pronolagus* (Nowak 1999) have a similar vocalization like *P. furnessi*. These results indicate that the small home range size and nocturnal activity of the rabbit are adapted to the steep slopes, and to the high temperature in this sub-tropical zone, respectively.

Decrease of Population and Conservation

The distribution and population size of the rabbit has recently decreased quite rapidly. The range size estimated by counting pellets during 1992–1994 was 334.7 km^2 (47% of the island) on Amami-Ohshima Island, and 33.0 km^2 (13% of the island) on Tokuno-Shima Island (Sugimura et al. 2000; Sugimura and Yamada 2004). The size of its range on Amami-Ohshima Island in 1994 was 20–40% smaller than those estimated in 1974 (Fig. 2). One fragmented population was recorded thereon. In addition, range size as estimated by pellet counts during 2002–2003 at 301.4 km^2 (42% of the island) on Amami-Ohshima Island (Sugimura and Yamada 2004).

Population size, estimated by pellet counts during 1992–1994, was 2,500–6,100 on Amami-Ohshima Island and 120–300 on Tokuno-Shima

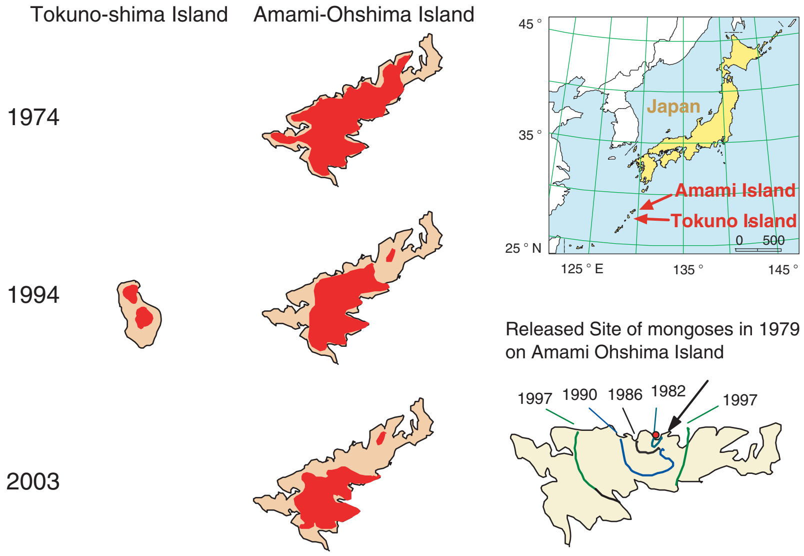

Fig. 2 Geographic distribution of *Pentalagus furnessi* in 1994 and 2003 compared with previous distributions (after Sugimura et al. 2000; Sugimura and Yamada 2004)

Island (Sugimura et al. 2000; Sugimura and Yamada 2004). Thus, the total number of Amami rabbits was 2,700–6,500. In addition, population size, estimated by pellet counts during 2002–2003, was 2,000–4,800 on Amami-Ohshima Island (Sugimura and Yamada 2004). So population size in 2003 on Amami-Ohshima Island was 20% lower than estimated in 1994.

The impact of habitat loss is quite severe for the Amami rabbit because extensive logging operations on the two islands have resulted in the area of old forests being reduced to less than 10–30% of its 1980 level (Sugimura et al. 2000). Furthermore, the impact of invasive predators such as feral dogs and cats on the Amami rabbit is also occurring in both islands. In addition, a severe affect on the Amami rabbit as well as on native species by the small Indian mongoose (*Herpestes javanicus*), introduced for expectation of poison snake control in 1979, has occurred on Amami-Ohshima Island since the 1990s (Yamada 2002). The mongoose, which invaded mountainous areas in the mid 1980s, has had a predatory impact on the native fauna living there. On the other hand, there has been no clear evidence that the mongooses prey on snakes (Yamada et al. 2000). During our diet analysis in mongoose living in Amami rabbit habitat, we found insects in 40% of 89 analyzed pellets, other invertebrate animals in 90%, amphibians and reptiles in 60%, mammals in 20%, and birds in 15% (Yamada et al. 2000). Eight percent of the pellets contained traces of Amami rabbit. Recent changes in the distribution and abundance of the Amami rabbit suggest that the mongoose has reduced Amami rabbit populations (Sugimura et al. 2000; Sugimura and Yamada 2004) (Figs. 2, 3).

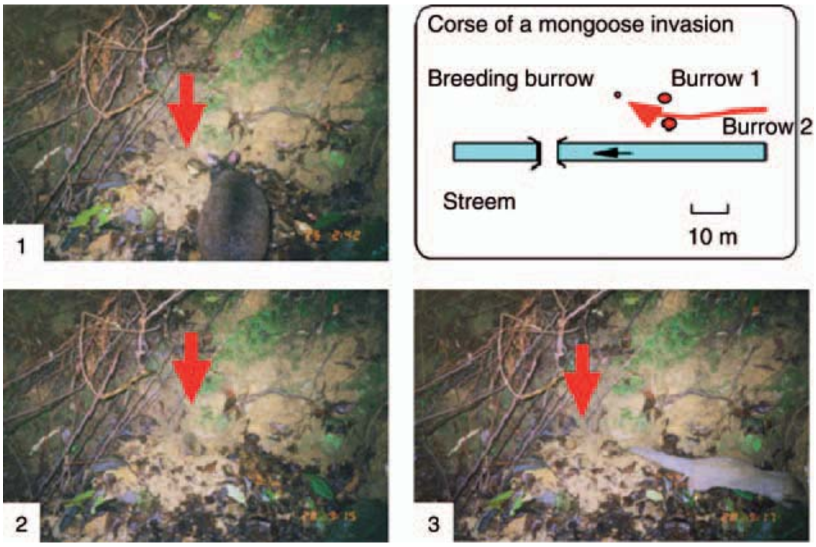

Fig. 3 A mongoose entering a breeding nest (*arrows*) of the endangered Amami rabbit, recorded by auto-sensor cameras on Amami-Ohshima Island during the full-scale project (Yamada and Sugimura 2004). A mother visited the breeding nest at 23:05 pm on December 25 and 02:42 am on December 26, 2002 (*1*). A mongoose entered into the breeding nest at 09:15 on December 28. The tail and hind foot of the mongoose were observed at the entrance of the nest (*2*). The mongoose left the nest at 09:17 on December 28 (*3*)

The Amami rabbit was designated a natural monument of Japan in 1921 (Uchida 1920) and upgraded to a special natural monument of Japan in 1963 by the government. Since this designation, hunting and capturing of the rabbit has been prohibited, but little attention has been paid to conservation, especially of its habitat. The Amami rabbit was listed as an endangered species, EN A2b, B1+2bce, C1, by the IUCN (2000), and ranked as an endangered species, EN(IB), by the Japanese government (Ministry of the Environment 2002).

For the conservation of the Amami rabbit and the whole ecosystem of both islands, it is necessary to take effective measures urgently to control exotic predators as well as habitat protection. The Lagomorph Specialist Group of the IUCN/SSC has also proposed conservation action needed for *P. furnessi*, among other lagomorphs; habitat conservation and management, control of introduced species, and research and monitoring programs (Chapman et al. 1990; Smith 2008, this book). In Amami-Ohshima Island, the Center for Conservation of Amami Wildlife of the Ministry of the Environment was established in 1999. The Amami rabbit was designated an endangered species by the Endangered Species Act by the Ministry of the Environment (2004). A new program of mongoose eradication was restarted in 2005 by the Ministry of the Environment to protect the ecosystem of the island, which includes the Amami rabbit and other native species (Yamada 2002; Yamada and Sugimura 2004).

Conclusions

The Amami rabbit is a symbol species living in damp subtropical forests of the Amami-Ohshima and Tokuno-Shima Islands. Whether we can protect this species and its habitat is one of most important issues confronting researchers. The Amami rabbit is a unique relic species that has some characteristic to adapt to damp subtropical forests. Further research and monitoring programs are necessary as well as the control of invasive species and habitat conservation to protect this rabbit.

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Concluding Remarks

Overview of Lagomorph Research: What we have learned and what we still need to do

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Palaeontology and Evolution

Lagomorphs can today be found on all continents, however, the present worldwide distribution does not reflect the relatively recent geographical expansion. The vast palaeontological data shows that lagomorphs are indeed an ancient taxonomic group and probably had (and still have) a great ecological importance. Some living genera lack fossil records, but there are a lot that only have been recorded with fossil data (Lopez-Martinez 2008, this book). It shows a large dynamic process in lagomorph evolution and morphological proximity among several lagomorph species. This complexity has resulted in intensive discussions among palaeontologists up to today (e.g. Erbajeva 2005; Lopatin and Averianov 2006). However, there are still some important open questions, such as: when and where does the common ancestral lagomorph appear, in Asia or America? Did the main lagomorph groups of ochotonids and leporids evolve independently? Why are there important time lags between the first arrival and definitive settlement in some areas, e.g. Europe? How can we solve some of the taxonomic uncertainties in the fossil data of some groups, e.g. *Lepus*? What is the closest taxonomic group to lagomorphs; and how can we calibrate the molecular data with paleontological data?

The last two questions are indeed very current, both for genetic and palaeontological studies. Lagomorphs are usually considered as being close to rodents (forming the *Glires* group), but not everyone agrees (see e.g. Douzery and Huchon 2004). In the last two decades, primarily due to the exploding increase in genetic studies, a great number of papers have appeared assembling molecular phylogenies (e.g. Halanych et al. 1999; Niu et al. 2004; Wu et al. 2005; Robinson and Matthee 2005). In some cases, these molecular phylogenies clarify some of the puzzling taxonomic situations, but in others they are not congruent with fossil data, causing even more confusion. We know today that each molecular set of markers tells us one story and only the combination of several

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markers would eventually clarify some situations (Alves et al. 2006; Ferrand 2008; Suchentrunk et al. 2008, both this book).

On the other hand, although these studies constitutes the most comprehensive intergeneric study on leporids, showing the monophyly of 11 genera, there are still several intrageneric questions to be resolved. A good example is found in the genus *Sylvilagus*. Somewhat surprisingly, a comprehensive molecular phylogeny that includes the majority of cottontail species is still lacking. Phylogenetic relationships among pikas are also not well resolved. Yu et al. (2000) reported the phylogeny of the genus *Ochotona* using mtDNA sequences and suggests a division into three subgenera. However, this study only includes 19 *Ochotona* species and a more recent comprehensive study by Niu et al. (2004) thus does not support this view. In addition, as mentioned previously, molecular phylogenies based solely on one mitochondrial characteristic should be avoided, and thus we consider that the evolutionary processes of *Ochotona* are also poorly understood.

Apart from phylogenetic aspects, other fields of genetics should be stressed, too. Several studies have been conducted on lagomorph population genetics. However, most of these studies have been done on the European hare and on the European rabbit. As mentioned in this book (Ferrand 2008, this book), the European rabbit population genetic studies have made achievable preposterous inferences about the population history of this species as well as important assumptions for other molecular and ecological studies. On the other hand, the European hare genetic studies also give important information about ecological features and evolutionary adaptations. In general, the available data shows a remarkable gene flow, even over long-distances, resulting, in a large panmictic population of European hares in Central Europe (see e.g. Suchentrunk et al. 1998; Kasapidis et al. 2005). Also, the recent genetic papers show that hybridisation on hares is not that uncommon. Introgressive hybridisation was initially demonstrated in Sweden between the Mountain hare and the introduced European hare (Thulin et al. 1997). More recently, it has been shown that it also occurred in the past between the Mountain hare and other hare species in the Iberian Peninsula (Alves et al. 2003). This past and ongoing hybridization have been confirmed in several other geographical regions and between different hare species (Thulin et al. 2006; Alves et al. 2003; Melo-Ferreira et al. 2004, 2005). Taken together, there is an urgent need to strengthen the cooperation between groups working on genetics and palaeontology in order to produce holistic views on the evolution in lagomorphs.

Population Ecology and Dynamics

Basic data on population ecology (e.g. rates for mortality of fecundity) are crucial for the development of sound strategies in the conservation and management of lagomorphs (see Morrison and Hik 2008; Rödel and von Holst 2008, this book). As stressed by various authors in this book, we know nothing or very little about the biology or ecology of numerous lagomorph species. In some cases, like the recently described Annamite Striped rabbit (*Nesolagus timminsi*) we know only the potential distribution (Can et al. 2001; Smith 2008a, this book). In other species like the Omilteme rabbit (*Sylvilagus insonus*), which has been described long ago, only fragments of its biology and ecology have been uncovered. To date, we do not know if this species even still exists. In contrast, we have comprehensive knowledge about one of the best-studied lagomorph species, the Snowshoe hare (*Lepus americanus*). This leporid has been studied over the last 75 years in order to understand the well-known 10-year population cycle which is mentioned in standard textbooks in ecology (summarized in Krebs et al. 2001). However, due to new methods and insights, new factors affecting the population dynamics are still being revealed, among them climate change (Stenseth et al. 2004) and forest fires (Ferron and St-Laurent 2008, this book). Hence, a lot of work remains to be done in order to fully understand the ecology of this species. In parallel, ecologists should begin to focus on those lagomorphs that are rare or have been only poorly studied (before they get extinct).

As mentioned above, global climate change also affects the 10-year cycle of Snowshoe hares. However, global climate change might also have a strong impact on a number of lagomorphs, especially those with a tiny ecological niche. For example, the Mountain hare (*Lepus timidus*) and its subspecies are affected by the warming phase both in the glacial refugees (e.g. the Alps) as well as in their northern ranges (Scandinavia). In both areas, potential habitat is declining and the European hare (*Lepus europaeus*) replaces its relative, either through competition or genetic introgression (Thulin 2003, see above). This will reduce the distribution range and thus endanger isolated subspecies (e.g. *Lepus timidus varronis*). Hence, there is a need for further studies about the ecological flexibility in lagomorph species, especially regarding adaptability to changing environments.

One adaptation of some lagomorph species to seasonal climate change is the change in fur colour from brown to white in autumn. This strategy camouflages Mountain hares and other Palaearctic lagomorphs when their habitat gets covered with snow, but it could be dangerous for them when there is a delay in snowfall or less snowfall than normal. One species of Mountain hare, the Irish hare *Lepus timidus hibernicus*, does not change fur colour, which seems to be an evolutionary stable strategy as snow in Ireland is rather rare (Dingerkus and Montgomery 2002). However, we hypothesise that climate change should result in a delayed moult or even no change at all in the fur colour in these species. It will be the task of ecological genetics and ecophysiology to discover the mechanisms related to these aspects and their plasticity.

Even more pronounced in their potential vulnerability to climate changes are pikas that live in cold environments and have high body temperatures (see Smith 2008b, this book). In this case, even small changes in the mean annual temperature might not only change the habitat (vegetation, moisture) but also result in heat stress. To sum up, climate change might have various effects on several lagomorph species and we need basic data on their ecology to understand the potential impact of changes in temperature and precipitation on their population dynamics.

Physiology and Behaviour

Studies on lagomorph physiology are often conducted in the lab. Here, we get detailled insights into fundamental aspects of lagomorph biology, such as mother-young-relationships (Schaal et al. 2008; Bautista et al. 2008, both this book) or reproductive energetics (Hackländer et al. 2002a, b). In the field , however, many lagomorph studies (like Stott et al. 2008, this book) describe physiological phenomenona which call for a closer combination of field and laboratory work. Similar prominent examples for this are caecotrophy and superfetation. Lagomorphs are known to produce two types of faeces: soft and hard. Both are re-ingested; the soft ones obligatory (caecotrophy) and the hard ones facultative (coprophagy). This re-ingestion enables lagomorphs to use the available diet more efficiently and to live in habitats with poor food and nutrient availability. It is believed that this phenomenon, which is quite rare in other mammalian taxa, is the reason for the current success of lagomorphs (Hirakawa 2001). Although we understand the mechanical processes in the gut that are responsible for the separation of food particles and the production of soft and hard faeces, we are not aware of the physiological impact of caecotrophy (Hirakawa 2001). We know that lagomorphs will die if they are prevented from re-ingesting their soft faeces and we also know that these soft faeces contain a high amount of nutrients, but we do not know why. It could be the presence of bacteria in the caecum or just the nutrient concentration process. When we discover the physiological impact of caecotrophy we can start to reach insights into its role for lagomorph ecology.

Another biological phenomenon that can be found at least in the genus *Lepus* is superfetation: female hares can have an ovulation while they are pregnant leading to two litters of different age in one uterus. This is quite unique, but superfetation has been described for hares since the classical philosopher Herodot (Zörner 1990). Several studies showed that it is common in captivity (e.g. Hediger 1948), but rather rare in the field (Flux 1967). Therefore, it has been discussed thoroughly whether superfetation in hares is just pathological (like it is humans) or an important reproductive strategy: Using superfetation hares are able to shorten interbirth interval and to produce more litters per year. To clarify the open questions on superfetation, studies on the mechanisms behind it and its pros and cons are mandatory. With this, we would be able to understand why it is so rare in the field or whether superfetation depends on other extrinsic factors such as population density or costs for gestation and lactation.

This book provided an excellent example of interspecific competition in two prominent lagomorph species (Flux 2008, this book). Often this competition is followed by translocations of lagomorphs for restocking of introduction (see Masetti and De Marinis 2008, Letty et al. 2008 or Rosin et al. 2008; Williams et al. 2008, all this book). However, translocations of individuals are often accompanied by translocations of diseases and alien genes, both potential threats to rare subspecies or species (e.g. Litvaitis et al. 2008, this book). In addition, autochthonous lagomorph species may be suppressed and expelled by the dominant behaviour of allochthonous ones (see Rosin et al. 2008, this book). Nevertheless, while DNA markers and antibodies are easily and quickly detectable, behavioural strategies of mammals have to be revealed by extensive observational studies. Even in common lagomorph species there is still a lack of information about space use, dispersal, sociality, and other aspects. Improvements in telemetry and mark-recapture-techniques may help to fill this gap and thus to develop sustainable strategies for successful restocking (Letty et al. 2008, this book) or pest control (Mutze et al. 2008, this book).

Interestingly, many of the translocated lagomorphs show a high adaptability to a huge number of various habitats. In general, lagomorphs can be found in almost all environments, from desert to arctic, as well as in different latitudes and longitudes. Although most of the species are well adapted to different regional environmental conditions (like the Swamp rabbit, Snowshoe hare, and other examples), some forms have a remarkable ecological plasticity, occurring in very different kinds of habitat. A good example of this is the European rabbit (*Oryctolagus cuniculus*). Notwithstanding this species is original from the Iberian Peninsula, it can be found in desert regions as well, like in Morocco, and in completely arctic areas, like Macquarie Island. This makes the European rabbit by far the most successful colonizing lagomorph (Long 2003), which is even able to improve its own habitat (Gálvez et al. 2008, this book). Another classical example is the European hare. Although this species is very common in continental climates in central Europe, it can also be found in subdesert (Israel), subtropical (Argentina), and in subarctic (Scandinavia) habitats. Why some species are so adaptable to different habitats and others are restricted to some small regions is an open question that should be explored in future works.

Diseases

Lagomorphs and their diseases are studied to understand basic backgrounds of important pathogens (Frölich and Lavazza 2008; Lavazza and Capucci 2008, both this book), to improve restocking processes (Letty et al. 2008, this book) and to use pathogens to manage lagomorph pest species (Mutze et al. 2008, this book). It is evident in all three fields of research, that lagomorphs play an important role as vectors of many diseases. There are several cases of described zoonoses, namely tularaemia, leptospirosis, brucellosis (Winkelmayer et al.

2005), toxoplasmosis (Sroka et al. 2003), encephalitozoonosis (Smielewska-Los et al. 2004) and mycosis (Nakamura 2003). Among them, tularaemia might be the predominant zoonosis, as infected humans are more abundant in areas where infected wildlife had been recorded. Apart from that, even considered anecdotal, there are several other reports of zoonoses between lagomorphs and humans. For instance, Scaife et al. (2006) recently described that several visitants of a natural park in Norfolk (UK) became infected with verocytotoxin originating from *Escherichia coli* (VTEC) 0157, which was associated with European rabbit faeces. In addition, lagomorphs can also be responsible for several diseases that affect domestic animals like sheep or dogs.

Interestingly, diseases and parasites have often been neglected in recent reviews for population dynamics in lagomorphs (e.g. Smith et al. 2005). However, several studies provide evidence for important impacts of diseases, e.g. in the Mountain hare (Newey et al. 2005). Knowledge about the causes of population increases or decreases will increase in the future if ecologists and veterinarians would combine their work more intensively.

Conservation and Management

In the first view, lagomorphs are everywhere, extremely abundant and widespread, and can become a pest, thus not requiring a conservation status. However, this is true only for a few species in very specific cases, for example the European rabbit in Australia (Cooke 2008, this book). Indeed, approximately a quarter of lagomorph species are threatened with extinction, which represents about 18 species (see Alves and Hackländer 2008, this book). As outlined in Smith (2008a, this book), during the last years the number of threatened lagomorphs increased because (1) the knowledge about some species increased, and (2) new species were described (e.g. *N. timminsi* in Vietnam). Since most of these species are locally adapted, representing evolutionary units of a specific region, the main conservation concern is associated with habitat perturbation, either from humans or from natural competitors (Farías et al. 2008; Yamada 2008, both this book). However, another important issue for the conservation status evaluation are monitoring surveys. This is a key point since we need to know population trends. Several improvements have been made in this respect, namely in developing census methods (such as using pellets or genetics methods), but there is a huge lack of information, namely in most of the *Ochotona*, *Lepus* and *Sylvilagus* species, as well as in several other genera like *Caprolagus*, *Nesolagus* and *Bunolagus*.

Interestingly, in the last few years another conservation problem has arisen. While European rabbits and European hares are widely distributed and very abundant in some areas, these species are endangered in some areas. For example, the European rabbit is decreasing in Spain and Portugal, resulting in the fact that European rabbit have been classified as near threatened in the

Portuguese Red Data Book (see also Smith 2008a, this book). As the European rabbit is the main prey of critically endangered predators (e.g. Iberian lynx, Ferrer and Negro 2004) the Spanish and Portuguese governmental institutions spend a large amount of money and effort to reverse the rabbit decline in the Iberian Peninsula. One strategy is to reintroduce rabbits from other areas (Letty et al. 2008, this book). However, there is an urgent need for detailed genetic studies to detect subspecies in order to avoid the loss of endangered subspecies by introduction of allochthonous subspecies. In general, taxonomic problems in lagomorphs are enlarged by the unclear situation of subspecies and their status (see above).

Finally, managing lagomorphs can range from conservation to pest control. Another aspect is hunting. The World Conservation Union, IUCN, recognised in its Policy Statement on Sustainable Use (IUCN 2000) that the use of wild living resources, if sustainable, is an important conservation tool because the social and economic benefits derived from such use provide incentives for people to conserve them. One form of use is hunting, and in fact, many lagomorph species are important game species (see Chapman and Flux 2008, this book). However, only few studies have been conducted to prove the sustainability of harvesting in lagomorphs or to model sustainable harvest rates (e.g. Marboutin et al. 2003). The lack of such models is due to missing data on mortality and fecundity rates of numerous lagomorphs (see above). Hence, the study of these issues is needed for both the general understanding of population dynamics as well as for the development of strategies for a sustainable wildlife management.

Conclusion

This book calls for an increase in lagomorph research, both on basic and applied aspects. We are convinced that different disciplines of lagomorph research have to intermingle in order to enhance the knowledge about this fascinating mammalian taxon. Hopefully, this book will help to turn the balance.

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Appendix

Lagomorph Species: Geographical Distribution and Conservation Status

Paulo C. Alves[1]* and Klaus Hackländer[2]

Lagomorph taxonomy is traditionally controversy, and as a consequence the number of species varies according to different publications. Although this can be due to the conservative characteristic of some morphological and genetic traits, like general shape and number of chromosomes, the scarce knowledge on several species is probably the main reason for this controversy. Also, some species have been discovered only recently, and from others we miss any information since they have been first described (mainly in pikas). We struggled with this difficulty during the work on this book, and decide to include a list of lagomorph species (Table 1). As a reference, we used the recent list published by Hoffmann and Smith (2005) in the "Mammals of the world" (Wilson and Reeder, 2005). However, to make an updated list, we include some significant published data (Friedmann and Daly 2004) and the contributions and comments of some lagomorph specialist, namely Andrew Smith, John Litvaitis, Terrence Robinson, Andrew Smith, Franz Suchentrunk, and from the Mexican lagomorph association, AMCELA. We also include summary information about the geographical range of all species and the current IUCN conservation status. Inevitably, this list still contains some incorrect information. However, a permanently updated lagomorph list will be provided via the *World Lagomorph Society* (www.worldlagomorphsociety.org).

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Table 1 List of Lagomorph species with the scientific and common name as well as geographical range and IUCN conservation status. The information was based on Hoffmann and Smith (2005), Friedmann and Daly (2004), and unpublished data. Conservation status is based on the information available in the "2004 IUCN red list of threatened species" (IUCN 2004).

Species	Common name	Local	IUCN status
Ochotona alpina	Alpine pika	Asia: Sayan and Altai Mountains; Khangai, Kentei; upper Amur drainage (NW Kazakhstan, S Russia, NW Mongolia); N Xinjiang (China).	LC
Ochotona argentata	Silver pika	Asia: Restricted the Helan Shan range, Ningxia (China).	CR
Ochotona cansus	Gansu pika	Asia: C China (Gansu, Qinghai, Sichuan); isolated populations in Shaanxi and Shanxi.	LC
Ochotona collaris	Collared pika	North America: WC Mackenzie, S Yukon, NW British Columbia (Canada); SE Alaska (USA).	LC
Ochotona curzoniae	Plateau pika	Asia: Tibetan plateau; adjacent Gansu, Qinghai, Sichuan (China), Sikkim (India) and E Nepal.	LC
Ochotona daurica	Daurian pika	Asia: Steppes from Altai, Tuva and Transbaikalia (Russia) through N China and Mongolia, south to Qinghai province (China).	LC
Ochotona erythrotis	Chinese red pika	Asia: E Qinghai, W Gansu, S Xinjiang, Tibet, and possibly N Sichuan (China).	LC
Ochotona forresti	Forrest's pika	Asia: NW Yunnan, SE Tibet (China); N Burma, Assam, Sikkim (India); Bhutan.	NT
Ochotona gaoligongensis	Gaoligong pika	Asia: only known from type locality – Dongsao-fang, Yunnan (China).	DD
Ochotona gloveri	Glover's pika	Asia: W Sichuan, NW Yunnan, NE Tibet, SW Qinghai (China).	LC
Ochotona himalayana	Himalayan pika	Asia: Mountain Jolmolungma (Everest), S Xizang (China); probably adjacent to Nepal.	LC
Ochotona hoffmanni	Hoffmann's pika	Asia: Restricted to the subalpine zone of Hentiyn Nuruu ridge, Bayan-Ulan Mountains (Mongolia); Erman range (Russia).	VU
Ochotona huangensis[1]	Tsing-Ling pika	Asia: Mountains of C China, including Shaanxi, Gansu, Qinghai and Sichuan provinces.	EN
Ochotona hyperborea	Northern pika	Asia: Ural, Putorana, Sayan Mountains, E of Lena river to Chukotka, Koryatsk and Kamchatka, upper Yenesei, Transbaikalia and Amur regions, Sakhalin Island (Russia); NC Mongolia; NE China; N Korea; Hokkaido (Japan).	LC

Table 1—Continued

Species	Common name	Local	IUCN status
Ochotona iliensis	Ili pika	Asia: only known in type locality – Tien Shan Mountain, Xinjiang (China).	VU
Ochotona koslowi	Kozlov's pika	Asia: Arkatag range, Kunlun Mountains, S shore of Aru-Tso lake, E of Lungdo, Ngari, Xizang (China).	EN
Ochotona ladacensis	Ladak pika	Asia: SW Xinjiang, Qinghai, E Tibet (China); Kashmir (India); Pakistan.	LC
Ochotona macrotis	Large-eared pika	Asia: Mountain regions including the Himalayas (Nepal, India) from Bhutan through Tibet, Qinghai, Xinjiang, Sichuan and Yunnan (China), Karakorum (Pakistan), Hindu Kush (Afghanistan), Pamir, and W Tien Shan Mountains (Kyrgyzstan, Tajikistan, SE Kazakhstan).	LC
Ochotona muliensis	Muli pika	Asia: only known from type locality Ting-Tung-Niu-Chang, Sichuan (China).	DD
Ochotona nigritia	Black pika	Asia: only known from type locality Piyanma, Yunnan (China).	NE
Ochotona nubrica	Nubra pika	Asia: Southern edge of Tibetan plateau from Ladak (India, China) through Nepal to E Tibet (China).	LC
Ochotona pallasi	Pallas's pika	Asia: Discontinuous in arid areas (mountains and high steppes) in Kazakhstan; Altai mountains, Tuva (Russia); Mongolia to Xinjiang, Inner Mongolia and Ningxia (China). With some isolated subspecies that are endangered: *O. p. hamica* and *O. p. sunidica*.	LC
Ochotona princeps	American pika	North America: Mountains of W North America from British Columbia (Canada) to N New Mexico, Utah, C Nevada and EC California (USA).	LC
Ochotona pusilla	Steppe pika	Asia: Steppes from middle Volga (Russia), through N Kazakhstan to upper Irtysh River and Chinese border. Not yet recorded in China.	VU
Ochotona roylei	Royle's pika	Asia: Himalayan mountains in NW Pakistan and India to Nepal; adjacent Tibet (China).	LC
Ochotona rufescens	Afghan pika	Asia: Afghanistan, Baluchistan (Pakistan), Iran, Armenia and SW Turkmenistan.	LC

(*Continued*)

Table 1—Continued

Species	Common name	Local	IUCN status
Ochotona rutila	Turkestan red pika	Asia: Isolated ranges from the Parmis (Tajikistan) to Tien Shan (SE Uzbekistan, Kyrgyzstan, SE Kazakhstan); perhaps N Afghanistan and E Xinjiang (China).	LC
Ochotona thibetana	Moupin pika	Asia: Shanxi, Shaanxi, W Hubei, Yunnan, Sichuan, S Tibet (China); N Burma; Sikkim (India); perhaps adjacent Bhutan.	LC
Ochotona thomasi	Thomas's pika	Asia: NE Qinghai, Gansu, and Sichuan (China).	NT
Ochotona turuchanensis	Turuchan pika	Asia: From middle to lower Yenesei river eastward to middle Lena river and lake Baikal; the middle Siberian plateau and adjacent Lena river basin (Russia).	NE
Prolagidae			
Prolagus			
Prolagus sardus	Sardinian pika	Europe: Mediterranean isles of Corsica (France) and Sardinia (Italy).	EX
Leporidae			
Brachylagus			
Brachylagus idahoensis	Pygmy rabbit	North America: SW Oregon to EC California, SW Utah, N to SW Montana (USA). Isolated population in WC Washington (USA).	NT
Bunolagus			
Bunolagus monticularis	Riverine rabbit	Africa: C Karoo, Cape colony (South Africa).	CR
Caprolagus			
Caprolagus hispidus	Hispid hare	Asia: S Himalaya foothills from Uttar Pradesh (India) through Nepal and West Bengal to Assam (India), and south through NW Bangladesh.	EN; CITES (I)
Lepus			
Lepus alleni	Antelope jackrabbit	North and Central America: SC Arizona (USA) to Nayarit and Tiburon Island (Mexico).	LC

Table 1—Continued

Species	Common name	Local	IUCN status
Lepus americanus	Snowshoe hare	North America: S and C Alaska (USA), from Hudson Bay to Newfoundland (Canada); Appalachians, S Michigan, North Dakota, MC New Mexico, SC Utah and EC California (USA); introduced in some islands, e.g. Anacosti (Canada).	LC
Lepus arcticus[2]	Arctic hare	North America: Greenland and Canadian artic islands southward in open tundra to shore of Hudson Bay, thence northwest of Fort Anderson on coast of Arctic Ocean. Isolated populations in tundra of North Quebec and Labrador, and on Newfoundland (Canada).	LC
Lepus brachyurus	Japanese hare	Asia: Honshu, Shikoku, Kyushu, Oki and Sado Islands (Japan).	LC
Lepus californicus	Black-tailed jackrabbit	North and Central America: Hidalgo and S Queretaro to N Sonora and Baja California (Mexico); north to SW Oregon and C Washington, S Idaho, E Colorado, S South Dakota, W Missouri, and NW Arkansas (USA). Apparently isolated population in SW Montana (USA).	LC
Lepus callotis	White-sided jackrabbit	North and Central America: C Oaxaca (Mexico), north discontinuously to SW New Mexico (USA). Range allopatric with *L. alleni*, to which it is probably related.	NT
Lepus capensis[3]	Cape hare	Africa and Asia: South Africa, Namibia, Botswana, Zimbabwe, S Angola, S Zambia, Mozambique; Tanzania, Kenya, Somalia, Ethiopia, countries of the Sahel and Sahara and N Africa; thence eastward through the Sinai to the Arabian Peninsula, Jordan, S Syria, S Israel and W and S Iraq, W of the Euphrates River.	LC
Lepus castroviejoi	Broom hare	SW Europe - Iberian Peninsula: Restricted to the Cantabrian Mountains in N Spain.	VU
Lepus comus	Yunnan hare	Asia: Yunnan, W Guizhou (China).	LC
Lepus coreanus	Korean hare	Asia: Korea; K Kirin, S Liaoning, E Heilungjiang (China).	LC

(*Continued*)

Table 1—Continued

Species	Common name	Local	IUCN status
Lepus corsicanus	Corsican hare	Europe: S Italy, from the Abruzzo mountains southwards; Sicily (Italy); introduced in Corsica. (Formerly included in *L. capensis* and *L. europaeus*)	NE
Lepus europaeus	European hare	Europe and Asia: throughout Europe, from Sweden and Finland to Britain, and from N Iberian Peninsula to W Siberian lowlands (Russia); South to N Israel, N Syria, the Tigris-Euphrates valley and W Iran. SE border of range (Iran) from S Caspian Sea south to Persian Gulf. Introduced in several regions, like South America, Australia, New Zealand and several islands including Barbados, Reunion and Falklands.	LC
Lepus fagani	Ethiopian hare	Africa: N and W Ethiopia, and adjacent SE Sudan, south to extreme NW Kenya.	DD
Lepus flavigularis	Tehuantepec jackrabbit	North America: Coastal plains and bordering foothills on south end of Isthmus of Tehuantepec, Oaxaca (Mexico), along Pacific coast to Chiapas (Mexico). (Now restricted to small area between Salina Cruz, Oaxaca and extreme W Chiapas).	EN
Lepus granatensis	Granada hare	SW Europe: Iberian Peninsula, except NE Spain; Mallorca (Balearic Islands, Spain).	NE
Lepus habessinicus	Abyssinian hare	Africa: Djibouti, E Ethiopia, Somalia, perhaps NE Kenya.	LC
Lepus hainanus	Hainan hare	Asia: lowlands of Hainan Island (China).	VU
Lepus insularis	Black jackrabbit	North America: Restricted to type locality – Espiritu Santo Island, Gulf of California - Mexico.	NT
Lepus mandshuricus	Manchurian hare	Asia: Ussuri region (Russia); NE China; extreme NE Korea.	LC
Lepus microtis[4]	African Savanna hare	Africa: from Atlantic coast of NW Africa (Senegal, south to Guinea and Sierra Leone) eastwards across Sahel to Sudan and extreme W Ethiopia; southward through E Africa (E Republic of Congo, W Kenya) to NE Namibia, Botswana and KwaZulu-Natal (South Africa).	LC

Table 1—Continued

Species	Common name	Local	IUCN status
Lepus nigricollis	Indian hare	Asia: Pakistan, India, Bangladesh, except Sunderbands; Sri Lanka; introduced into Java and Mauritius, Gunnera Quoin, Anskya, Reunion and Coisin Islands in the Indian Ocean. (Considered native to Java by some authors).	LC
Lepus oiostolus	Woolly hare	Asia: Tibetan plateau, from Ladak to Sikkim (India), Nepal, and eastwards through Xizang (Tibet) and Qinghai, Gansu and Sichuan (China).	LC
Lepus othus[2]	Alaskan hare	North America and Asia: W and SW Alaska (USA); E Chukotsk – Russia.	LC
Lepus peguensis	Burmese hare	Asia: C, S Burma from Chindwin River valley east to through Thailand; Cambodia; S Laos; S Vietnam; south in upper Malay Peninsula (Burma, Thailand).	LC
Lepus saxatilis	Scrub hare	South Africa: South Africa and S Namibia.	LC
Lepus sinensis	Chinese hare	Asia: SE China from Yangtze river southward; Taiwan; disjunct in NE Vietnam.	LC
Lepus starcki	Ethiopian Highland hare	Africa: central highlands of Ethiopia.	LC
Lepus tibetanus	Desert hare	Asia: Afghanistan and Baluchistan eastwards through N Pakistan and Kashmir to the E Pamir, NW Xinjiang and Altai Mountains, then eastward across S Mongolia to Gansu and Ningxia (China). Some populations are sympatric to *L. tolai*.	NE
Lepus timidus	Mountain hare	Europe and Asia: Palaearctic from Scandinavia to E Siberia, except E Chukotsk (Russia), south to Sakhalin and Sikhote-Alin Mountains (Russia); Hokkaido (Japan); Heilungjiang, N Xinjiang (China); N Mongolia, Altai, N Tien Shan mountains; N Ukraine; E Poland and Baltics; isolated populations in the Alps, Scotland, Wales and Ireland.	LC
Lepus tolai	Tolai hare	Asia: Steppes north of Caspian Sea southwards along eastern shore of Caspian to E Iran: eastwards through Afghanistan; Kazakhstan and S Siberia, middle Asian republics to Mongolia; W, C and NE China.	NE

(*Continued*)

Table 1—Continued

Species	Common name	Local	IUCN status
Lepus townsendii	White-tailed jackrabbit	North America: C Alberta and Saskatchewan east to extreme SW Ontario (Canada), S to SW Wisconsin, Iowa, NW Missouri, west through C Kansas to NC New Mexico, west to C Nevada, EC California (USA) and north to SC British Columbia (Canada).	LC
Lepus yarkandensis	Yarkand hare	Asia: Steppes of Tarim basin, S Xinjiang (China), around edge of Takla Makan Desert.	NT
Nesolagus			
Nesolagus netscheri	Sumatran rabbit	Asia: Sumatra (Indonesia).	CR
Nesolagus timminsi	Annamite striped rabbit	Asia: Know only in the type locality – Vietnam, Ha Tinh province, Huong Son District.	DD
Oryctolagus			
Oryctolagus cuniculus	European rabbit	Europe: Originally from the Iberian peninsula, but introduced in all continents and in more than 800 islands, from desert (North Africa) to cold and arctic areas (like Macquarie Islands in the Artic Ocean). Considered a pest in several regions where it has been introduced (e.g., Australia and New Zealand).	LC
Pentalagus			
Pentalagus furnessi	Amami rabbit	Asia: Amami Island (Amami-Oshima, Ryukyu Island) - S Japan.	EN
Poelagus			
Poelagus marjorita	Bunyoro rabbit	Africa: S Sudan, Uganda, Burundi, NE Dem. Rep. Congo, Central African Republic, S Chad, disjunct population in Angola.	LC
Pronolagus			
Pronolagus crassicaudatus	Natal red rockhare	Africa: SE South Africa; extreme S Mozambique.	LC
Pronolagus randensis	Jameson's red rockhare	Africa: two disjunct areas: NE South Africa; E Botswana to extreme W Mozambique; Zimbabwe; and W Namibia, perhaps SW Angola.	
Pronolagus rupestris	Smith's red rockhare	Africa: two disjunct areas: S and C South Africa, S Namibia; and Africa, from N Malawi and E Zambia north C Tanzania to SW Kenya.	LC

Table 1—Continued

Species	Common name	Local	IUCN status
Pronolagus saundersiae[5]	Hewitt's red rock rabbit	Africa: South Africa, Lesotho and Swaziland.	LC
Romerolagus			
Romerolagus diazi	Volcano rabbit	North America: S Distrito Federal, E Estado de Mexico, W Puebla, and NE Morelos (Mexico).	EN; CITES (I)
Sylvilagus			
Sylvilagus aquaticus	Swamp rabbit	North America: S Illinois and SW Indiana, SW Missouri to SE Kansas southward through extreme W Kentucky and W Tennessee to E Oklahoma, E Texas, Louisiana, Alabama, Mississippi and NW South Carolina (USA).	LC
Sylvilagus audubonii	Desert cottontail	North and Central America: NE Puebla and W Veracruz (Mexico) to NC Montana and SW North Dakota, NC Utah, C Nevada, and NC California (USA), south to Baja California and C Sinaloa (Mexico).	LC
Sylvilagus bachmani	Brush rabbit	North and Central America: W Oregon (USA) S of Columbia River to Baja California (Mexico), E to Cascade-Sierra Nevada range (USA).	LC
Sylvilagus brasiliensis	Tapeti	North to South America: S Tamaulipas (Mexico) southward as far as Peru, Bolivia, N Argentina and S Brazil.	LC
Sylvilagus cognatus	Manzano Mountain cottontail	North America: restricted to the Manzano Mountains, New Mexico (USA).	NE
Sylvilagus cunicularius	Mexican cottontail	North America: S Sinaloa to E Oaxaca and Veracruz (Mexico).	NT
Sylvilagus dicei	Dice's cottontail	Central America: SE Costa Rica, NW Panama.	EN
Sylvilagus floridanus	Eastern cottontail	Central and North America: N, C and W Venezuela and adjacent to Columbia through USA; to NE Mexico, Arizona, north and east to North Dakota, Minnesota, N Michigan, New York and Massachusetts, Atlantic Coast south and Florida Gulf Coast (USA) west to Mexico, also S Saskatchewan, S Ontario and SC Quebec (C Canada).	LC
Sylvilagus graysoni	Tres Marias cottontail	North America: Tres Marias Islands, Nayarit (Mexico).	EN

(*Continued*)

Table 1—Continued

Species	Common name	Local	IUCN status
Sylvilagus insonus	Omilteme cottontail	North America: Appears restricted to Sierra Madre del Sur, C Guerrero (Mexico).	CR
Sylvilagus mansuetus	San Jose Brush rabbit	North America: Know only from the type locality – San Jose Island, Gulf of California (Mexico). (An insular allospecies closely related to *S. bachmani*).	NT
Sylvilagus nuttallii	Mountain cottontail	North America: Intermountain area in North America from S British Columbia to S Saskatchewan (Canada), south to E California, Nevada, C Arizona, and NW New Mexico (USA).	LC
Sylvilagus obscurus	Appalachian cottontail	North America: N Pennsylvania south and west the Appalachian mountains to N Alabama (USA).	LC
Sylvilagus palustris	Marsh rabbit	Northern America: Florida to SE Virginia (USA).	LC
Sylvilagus robustus	Robust cottontail	North America: Chisos, Davis and Guadalupe Mountains of Texas and New Mexico (USA), and C Sierra de la Madera and Sierra del Carmen of adjacent Coahuila (Mexico).	NE
Sylvilagus transitionalis	New England cottontail	North America: regenerating forests and native scrublands from southwestern Maine to southeastern New York, east of Hudson River (USA).	VU
Sylvilagus varynaensis	Venezuelan Lowland rabbit	Central America: Presently known only from the states of Barinas, Guarico and Portuguesa (Venezuela).	NE

EX – Extinct; CR – Critically endangered; EN – Endangered; VU- Vulnerable; NT – Near threatened; LC – Least concern; DD – Data deficient; NE – Not evaluated. CITES I - in the appendix I of CITES

[1] Recent molecular data clearly indicate independent species from *O. thibetana*

[2] Accordingly to some authors *L. arcticus* and *L. othus* are might be *L. timidus* subspecies.

[3] Polytypic species with a great controversy in its taxonomy and geographical distribution. Might include *L. europaeus*

[4] Some authors consider as *L. victoriae*

[5] Recently distinguished from *Pronolagus rupestris* based on morphological and genetic differences

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